Molekulárně-genetické aspekty maligních komorových arytmií a náhlé srdeční smrti

Habilitační práce

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Obsah

Abstract

There is clear evidence that malignant ventricular arrhythmias (and thus sudden cardiac death) are genetically determined. Possibilities for prediction of the event are poor. The thesis consists of 17 included original articles of which the applicant is author or co-author. These articles are dealing with various aspects of genetics of sudden cardiac death pathophysiology, they are completed with connecting commentaries.

The genetic aspects of ventricular arrhythmias were studied in patients with hereditary arrhythmic syndromes (s. c. channelopathies) which serve as a clinical model of arrhythmogenesis. Groups of patients with congenital long QT syndrome and catecholaminergic polymorphic ventricular tachycardia were characterized clinically, various aspects of clinical diagnosis were analyzed. Mutation analysis of related genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, RyR2) were performed in all these patients with subsequent genotype-phenotype analyses.

Mutation analysis of above mentioned genes was performed also in patients with structural heart disease – coronary artery disease. The prevalence of selected, rare coding variants in was significantly higher in ventricular fibrillation survivors versus controls, confirming a mechanistic role for these genes among a selected subgroup of patients.

Despite being promising, these results cannot be used in risk stratification of sudden cardiac death. The possible SCD susceptibility alleles are likely to be as prevalent in noncoding regions of the genome as in coding sequences. Identification of such alleles will be a task for genome-wide association studies and the new emerging technologies developed for mass DNA sequencing. Any future genetic screening for SCD risk stratification should involve evaluation of large numbers of genomic variants in many pathways, each of which may be relevant to the risk of arrhythmias.

In all the included articles the applicant was either the first author or he was the cooperation coordinator of the involved departments and institutions. The applicant was also the principal investigator of majority the related research grants.

Předmluva

Tato habilitační práce je sestavena jako soubor 17 publikovaných prací, jejichž je uchazeč autorem či spoluautorem. Publikované práce jsou vloženy v PDF formátu, tedy v grafické podobě a jazyku odpovídajícím příslušnému časopisu. Jednotlivé články jsou uvedeny krátkými shrnujícími komentáři (je zmíněna případná hodnota impakt faktoru a počet citací ve Web of Science) a také propojeny doplňujícím textem, který je uvádí do kontextu se současným stavem znalostí.

Poděkování

Chtěl bych poděkovat oběma přednostům Interní kardiologické kliniky (IKK), bývalému, prof. MUDr. Bořivoji Semrádovi, CSc., který byl iniciátorem zahájení výzkumu hereditárních arytmických syndromů na našem pracovišti, i současnému přednostovi prof. MUDr. Jindřichu Špinarovi, CSc. za trvalou podporu.

Velký dík patří všem kolegům z IKK i dalších spolupracujících pracovišť, bez jejichž pomoci by realizace výzkumných projektů nebyla možná. Všichni jsou uvedeni jako spoluautoři některé z vložených publikací. Zvlášť chci poděkovat mé postgraduální studentce MUDr. Ireně Andršové, PhD, jejíž vklad byl po celou dobu jejího studia zcela zásadní.

1. Úvod

Náhlá srdeční smrt (NSS) je klinicky i společensky významným jevem. Tímto způsobem zmírá např. v USA asi 400 000 jedinců ročně. Etiologie, patofyziologie a riziková stratifikace NSS zatím nebyla uspokojivě vyřešena (1). Celá řada tzv. sofistikovaných rizikově-stratifikačních metod (variabilita tepové frekvence, senzitivita baroreflexu, analýza pozdních komorových potenciálů, atd.) nepřinesla zpřesnění predikce náhlé smrti. V rámci primární prevence tak zůstalo snížení ejekční frakce levé komory víceméně jediným kritériem, na základě kterého jsou pacientům implantovány finančně nákladné kardiovertery defibrilátory. Přitom v 5 letech od implantace využije tento přístroj jen asi 1/3 pacientů.

V posledních desetiletích byly publikovány výsledky několika geneticky zaměřených populačních studií (2, 3, 4, 5). V nich provedená multivariační analýza konvenčních rizikových faktorů pro ischemickou chorobu srdeční (ICHS) (faktory biologické, dietetické a faktory prostředí) ukázala, že pozitivní rodinná anamnéza náhlé srdeční smrti je silným nezávislým rizikovým faktorem NSS pro potomstvo. Anamnéza náhlého úmrtí u rodiče zvyšuje 1,8-krát riziko náhlého úmrtí pro potomka. V případě náhlého úmrtí obou rodičů pak relativní riziko pro potomstvo dosahuje překvapivě vysoké hodnoty 9,4. I když vezmeme do úvahy metodologická úskalí daného problému, podávají tyto studie přesvědčivý důkaz o familiárním výskytu NSS v běžné populaci (6). Při vědomí familiárně společně sdílených vnějších rizikových faktorů je zřejmá existence geneticky podmíněných odchylek různých fyziologických procesů, které zvyšují riziko NSS.

Genetická variabilita rizika náhlé smrti se může uplatňovat v následujících oblastech 1) procesy tvorby a propagace elektrického impulsu v myokardu, 2) procesy a faktory tvorby a stability aterosklerotického plátu, trombózy a ischémie v koronárním řečišti, 3) centrální i místní řízení excitability myokardu a cévní motoriky.

2. Hereditární arytmické syndromy jako model arytmické náhlé smrti

První jmenovaná oblast - procesy tvorby a propagace elektrického impulsu v myokardu – je dlouholetým předmětem výzkumu na našem pracovišti. V posledních desetiletích byla identifikována řada jednotlivých iontových proudů a jejich proteinových kanálů, které se podílejí na tvorbě akčního potenciálu srdečních buněk. U většiny z nich známe alespoň gen kódující jejich hlavní proteinovou podjednotku. Zásadní poznatky v této oblasti byly získány zejména při výzkumu relativně vzácných monogenně podmíněných arytmických syndromů -

především syndromu dlouhého QT intervalu (LQTS), Brugada syndromu a katecholaminergní polymorfní komorové tachykardie (CPVT). Ty jsou ve většině případů způsobeny mutací genu pro některý iontový kanál srdečních myocytů a tak představují model pro studium procesů arytmogeneze na molekulární úrovni.

V roce 1999 bylo popsáno několik případů "forme fruste" kongenitálního LQTS (7). V některých rodinách dosahovala penetrance plného vyjádření choroby jen 25%. Ostatní nositelé patologických mutací genů pro iontové kanály v těchto rodinách zůstávali zcela asymptomatičtí, tedy neměli ani prodloužený QT interval. Normální hodnota QT intervalu tak rozhodně nevylučuje, že konkrétní jedinec není nositelem potenciálně nebezpečné mutace.

2.1. Syndrom dlouhého intervalu QT

LQTS byl dlouho považován za vzácné onemocnění. V roce 2009 však byla publikována práce Schwartze *et al.* (8), kteří hodnotili EKG křivky u téměř 45 000 konsekutivních novorozenců v severní Itálii. Při dodržení poměrně přísných diagnostických kritérií zjistili výskyt syndromu 1:2500.

Novotný T. Syndrom dlouhého intervalu QT. Cor Vasa 2007;49(11):416-425.
(publikovaná přehledová práce)

Syndrom dlouhého intervalu QT*

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Novotný T (Interní kardiologická klinika, Fakultní nemocnice Brno, Brno, Česká republika). **Syndrom dlouhého intervalu QT.** *Cor Vasa 2007;49(11):416–425.*

Syndrom dlouhého intervalu QT je onemocnění charakterizované prodloužením intervalu QT a zvýšeným rizikem maligních arytmií, které mohou způsobit synkopu nebo i náhlou smrt. Typickým rysem je absence strukturálního onemocnění srdce. Článek shrnuje nejnovější poznatky o syndromu od „buňky až po lůžko": patofyziologii na molekulární i buněčné úrovni, klinickou diagnostiku a současné doporučené léčebné postupy. Krátce je zmíněn i sekundární syndrom dlouhého intervalu QT a další hereditární arytmické syndromy. Na závěr je uveden přínos studia syndromu dlouhého intervalu QT pro problém náhlé srdeční smrti v běžné populaci.
Klíčová slova: Gen – Iontový kanál – Náhlá srdeční smrt – Syndrom dlouhého intervalu QT

Novotný T (Department of Internal Medicine and Cardiology, Brno University Hospital, Brno, Czech Republic). **The long QT syndrome.** *Cor Vasa 2007;49(11):416–425.*

The long QT syndrome is characterized by QT interval prolongation and increased risk of malignant arrhythmias, which can cause syncope or even sudden death. A typical feature is absence of structural cardiac disease. The article summarizes the most recent information on the syndrome "from cell to bedside": pathophysiology at molecular and cellular levels, clinical diagnosis and the current recommended therapeutic approaches. The acquired long QT syndrome and other hereditary arrhythmic syndromes are also briefly mentioned. In conclusion, the contribution of long QT syndrome research to the issue of sudden cardiac death in the general population is emphasized.
Key words: Gene – Ion channel – Sudden cardiac death – Long QT syndrome

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ÚVOD

V posledním desetiletí došlo k obrovskému pokroku v chápaní membránových procesů v myokardu. Byla identifikována řada jednotlivých iontových proudů a jejich proteinových kanálů, které se podílejí na tvorbě akčního potenciálu. U většiny z nich dnes známe alespoň gen kódující jejich hlavní proteinovou podjednotku. Zásadní poznatky v této oblasti byly získány zejména při výzkumu relativně vzácných monogenně podmíněných arytmických syndromů – především syndromu dlouhého intervalu QT (LQTS). Jeho příčinou jsou ve většině případů mutace genů pro některý iontový kanál myokardu. Tento syndrom tak představuje přímé spojení mezi molekulární biologií a klinickou kardiologií a vytváří molekulární model pro studium procesů arytmogeneze.

HISTORIE

Historicky první zmínka o LQTS je z Norska. V roce 1957 Jervell a Lange-Nielsen[1] popsali rodinu se šesti dětmi, z nichž čtyři měly prodloužený interval QT, vrozenou hluchoněmost a trpěly recidivujícími synkopami. Tři z takto postižených dětí zemřely náhlou smrtí (Jervellův a Lange-Nielsenův syndrom). Počátkem 60tých let pak nezávisle na sobě Romano[2] a Ward[3] popsali podobný syndrom bez vrozené hluchoty (Romanův a Wardův syndrom). Etiologie zůstávala dlouho nejasná. V roce 1966 Yanowitz a spol.[4] prokázali, že interval QT může být prodloužen stimulací levého ganglion stellatum nebo pravostrannou stellektomií a tento poznatek byl poté využit v léčbě pacientů s LQTS. Navíc dal vznik hypotéze „sympatické dysbalance",[5] která za primární defekt považovala oslabenou pravostrannou sympatickou inervaci srdce. Zásadní průlom pro pochopení patofyziologie LQTS přinesl rok 1991, kdy tým genetiků z Utahu pod vedením M. T. Keatinga prokázal vazbu LQTS na lokus na 11. chromosomu.[6] V následujících letech pak byla prokázána vazba i na lokusy dalších chromosomů, v nichž byly poté identifikovány i konkrétní geny. Dnes tedy LQTS chápeme jako společné fenotypické vyjádření nejméně 8 geneticky odlišných nozologických jednotek *(tabulka I)*, jejichž příčinou jsou mutace genů kodujících jednotlivé podjednotky iontových

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Tabulka I
Základní typy LQTS ve vztahu ke genům a jejich produktům

Typ LQTS	Chromosom a lokus	Gen	Proteinový produkt
Jervellův a Lange-Nielsenův syndrom			
JLN 1	11p15.5	KCNQ1 (KVLQT1)	α I_{Ks}
JLN 2	21q22.1-22.2	KCNE1	β I_{Ks}
Romanův a Wardův syndrom			
LQT 1	11p15.5	KCNQ1 (KVLQT1)	α I_{Ks}
LQT 2	7q35-36	KCNH2 (HERG)	α I_{Kr}
LQT 3	3p21-24	SCN5A	α I_{Na}
LQT 4	4q25-27	ANK2	ankyrin
LQT 5	21q22.1-22.2	KCNE1	β I_{Ks} (minK)
LQT 6	21q22.1	KCNE2	β I_{Kr} (MIRP)

Jedinci s Jervellovým a Lange-Nielsenovým syndromem jsou homozygoti, s Romanovým a Wardovým syndromem heterozygoti

α, β – podjednotky iontových kanálů, I_{Ks} – pomalu se aktivující opožděný rektifikující K+ proud fáze 3 akčního potenciálu kardiomyocytů, I_{Kr} – rychle se aktivující opožděný rektifikující K^+ proud fáze 3 akčního potenciálu kardiomyocytů, I_{Na} – rychlý Na^+ proud fáze 0, ankyrin je cytoskeletární protein

membránových kanálů kardiomyocytů. Tyto kanály hrají důležitou roli v tvorbě a trvání akčního potenciálu. V důsledku jejich postižení dochází k rozvoji klinického obrazu LQTS.[7]

PATOFYZIOLOGIE LQTS

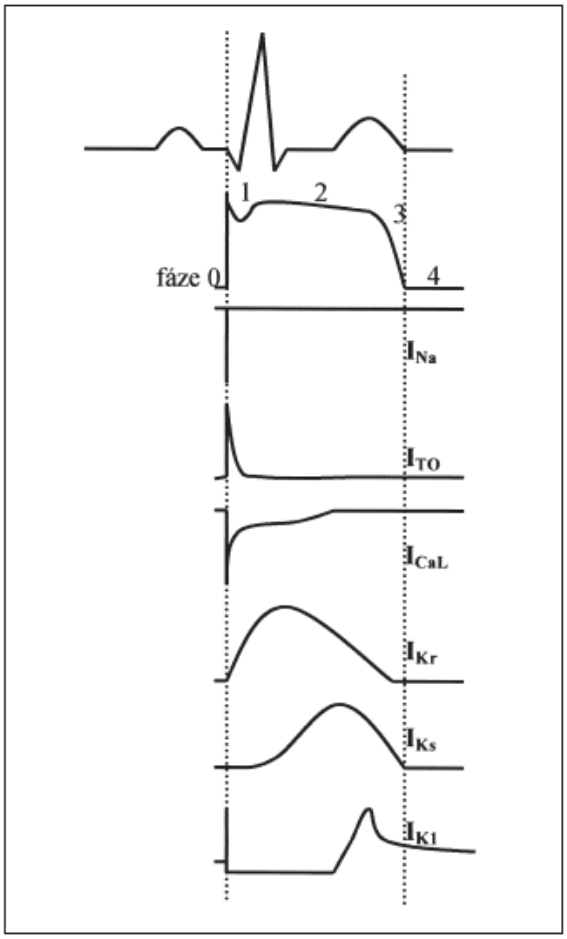
K pochopení souvislostí jednotlivých geneticky odlišných typů LQTS je vhodné zopakovat základní mechanismy vzniku a trvání akčního potenciálu srdečních myocytů.

Nejdůležitějšími ionty v tvorbě klidového i akčního potenciálu (AP) jsou kationty sodný (Na^+), draselný (K^+) a vápenatý (Ca^{2+}), které jsou po obou stranách membrány rozloženy nerovnoměrně, čímž vznikají napěťové gradienty. Na jejich udržení se podílí řada aktivních mechanismů. Tak v okamžiku klidového potenciálu je tedy vně buněčné membrány výrazná převaha Na^+ a Ca^{2+}, uvnitř pak převaha K^+. Po otevření iontových kanálů tečou příslušné iontové proudy ve směru těchto gradientů.

V procesech AP jsou důležité především tzv. „napěťově vrátkované" iontové kanály. To znamená, že se otevírají a zavírají v závislosti na aktuálním napětí na buněčné membráně, tedy vlastně v závislosti na přesunech iontů přes membránu. Tyto kanály jsou navíc vysoce selektivní pro konkrétní iont.

Tradičně se na AP popisuje 5 fází: fáze 0 – rychlá depolarizace, fáze 1 – časná repolarizace, fáze 2 – „plateau", fáze 3 – pozdní rychlá repolarizace, fáze 4 – klidový membránový potenciál *(obrázek 1)*.

Klidový membránový potenciál (méně než -80 mV) je vytvářen především činností Na^+/K^+ pumpy, dále pak jsou v této fázi otevřeny některé K^+ kanály. V okamžiku, kdy membránový potenciál činností pacemakerových center dosáhne -70 mV, otevírá se Na+ kanál. Výsledkem je mohutný ale velmi krátce trvající (asi 2 ms) proud sodíku do buňky (I_{Na}), vedoucí k depolarizaci buněčné membrány, na povrchovém EKG tomu odpovídá komplex QRS. Vzápětí se s různým zpožděním otevírají repolarizační, převážně K^+ kanály. Jako první je to tzv. „přechodný proud ven z buňky" – „transient outward" (I_{TO1} a I_{TO2}), výsledkem je fáze 2 – rychlý ale krátce trvající pokles AP. Zároveň se totiž začíná otevírat typ L Ca^{2+} kanálu (I_{CaL}),



Obr. 1 Povrchové EKG, akční potenciál srdečního myocytu a nejdůležitější iontové proudy

který po nějakou dobu brání dokončení repolarizace (fáze 2 – plateau). Mezitím ale nabývá na síle tzv. opožděný rektifikující K^+ proud (delayed rectifier – DR), který má v komorových myocytech 2 složky – rychle se aktivující DR (I_{Kr}) a pomalu se aktivující DR

(I_{Ks}). Především ty pak dokončují návrat membránového potenciálu ke klidovým hodnotám.[8,9]

Klidový i akční potenciál je tedy tvořen křehkou rovnováhou mezi iontovými proudy dovnitř a ven z buňky. Poškození kteréhokoli z nich může tuto rovnováhu narušit a vést tak ke změně trvání AP. Prodloužení AP a tím i prodloužení intervalu QT tedy teoreticky může být způsobeno jak zesílením nebo prodloužením depolarizačních proudů (tedy I_{Na} a I_{CaL}), tak oslabením či zkrácením repolarizačních proudů (především I_{TO1}, I_{Kr} a I_{Ks}).

Prodloužení trvání repolarizace, tedy prodloužení intervalu QT, usnadňuje vznik arytmií na podkladě tzv. časných následných depolarizací – „early after depolarizations" – kdy dochází ke znovuotevření depolarizujících kanálů (I_{Na} nebo I_{Ca}) ještě před ukončením repolarizace, tedy před „dokončením" AP. Vzniká tak nepravidelný sled rychle po sobě se opakujících depolarizací, které mohou na úrovni celého srdce způsobit polymorfní komorovou tachykardii typu „torsades de pointes". K tomuto předčasnému otevření výše uvedených kanálů dochází při takovém prodloužení repolarizace, které časově přesáhne dobu nutnou ke zotavení příslušného kanálu.[10] Proces může být dále usnadněn defektní inaktivací těchto kanálů. Jedna nebo i obě situace jsou přítomny u LQTS.

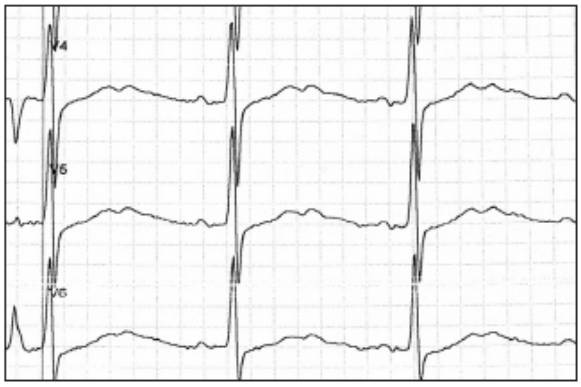
KLINICKÝ OBRAZ

Základními symptomy LQTS jsou prodloužení intervalu QT, korigovaného vzhledem k tepové frekvenci (QTc, nejčastěji podle Bazettovy formule $QTc = QT/RR^{1/2}$ s, kde QT je aktuální délka intervalu QT a RR je interval RR, obě hodnoty v sekundách), synkopy a náhlá smrt. Kombinace těchto příznaků je označována jako syndrom Romanův a Wardův. Je-li přítomna i vrozená hluchota, pak hovoříme o syndromech Jervellově a Lange-Nielsenově. V roce 1993 byly k těmto klasickým symptomům doplněny další *(tabulka II)*. Jejich souhrn je označován někdy též jako „Schwartzovo skóre", které přiřazuje každému ze symptomů určitou bodovou hodnotu. Výsledný součet pak určuje pravděpodobnost diagnózy.[11] Pacient, který získá 4 a více bodů má vysokou pravděpodobnost diagnózy tohoto syndromu, jeden a méně pravděpodobnost nízkou.

Tabulka II
Skóre pro diagnostiku LQT syndromu
(upraveno podle citace 11)

	Body
EKG nálezy	
A. QTc (podle Bazetta)	
> 0,48 s	**3**
0,46–0,47 s	**2**
0,45 s u mužů	**1**
B. Torsades de pointes	**2**
C. Alternans vlny T	**1**
D. Dvouvrcholová vlna T ve 3 svodech	**1**
E. Nízká tepová frekvence pro danou věkovou skupinu (u dětí)	**0,5**
Osobní anamnéza	
A. Synkopa	
při námaze	**2**
bez vazby na námahu	**1**
B. Vrozená hluchota	**0,5**
Rodinná anamnéza	
A. Jasná diagnóza LQTS u přímého příbuzného	**1**
B. Náhlé úmrtí přímého příbuzného před 30. rokem	**1**
Hodnocení	
≤ 1 bod – nízká pravděpodobnost diagnózy	
2–3 body – střední pravděpodobnost diagnózy	
≥ 4 body – vysoká pravděpodobnost diagnózy	

Prodloužení QTc je symptom, který dal celému syndromu jméno, a je-li přítomen, stává se základem diagnózy *(obrázek 2)*. Patologickou hranicí představuje po korekci k tepové frekvenci 460 ms (resp. 440 ms pro muže). Důležitým a překvapujícím zjištěním však byl záchyt nosičů patologické mutace s naprosto normální hodnotou QTc (z nichž někteří dokonce trpěli synkopami). Současné údaje ukazují, že až 1/3 nositelů patologických mutací může mít přinejmenším intermitentně normální hodnoty QTc.[12] Podobné zkušenosti máme i na našem pracovišti.[13] Důležitou roli hraje zřejmě i fakt, že okolnosti, za kterých může být patologická hodnota zachycena, se pravděpodobně liší u jednotlivých typů LQTS.



Obr. 2 Typický EKG obraz syndromu dlouhého intervalu QT – prodloužení intervalu QT a patologická dvouvrcholová vlna T (kdy druhý vrchol vlny T je vyšší než první vrchol), záznam s posunem 50 mm/s a voltáží 20 mm/mV

Byť je prodloužení intervalu QT vedoucí známkou syndromu, není jeho změření vždy jednoduché. Neexistuje konsenzus, ve kterém svodu vlastně interval QT měřit. V literatuře se stále traduje, že nejdelší interval QT je měřen obvykle ve svodu II. Tato metodika byla však již poměrně dávno zpochybněna.[14] Vhodnější metodou je měření v co nejvíce svodech současně, například Malik navrhuje alespoň měření ve svodech II a V2.[15]

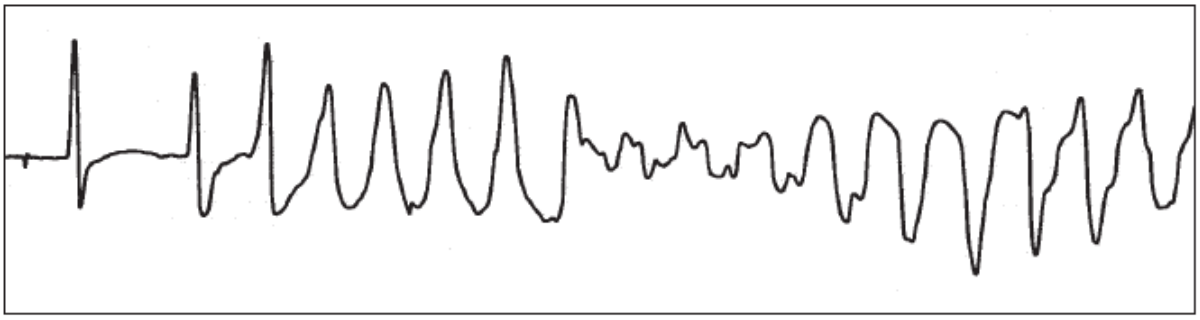
Problematické dále často bývá určení konce vlny T. V literatuře se traduje metoda, podle které je konec vlny T určen jako průsečík tečny proložené nejstrmější částí sestupného raménka s izoelektrickou linií.[16] Je však zřejmé, že článek Lepeschkina a Surawicze o této „tangenciální" metodě je mnohem častěji citován než pečlivě čten. Sami autoři totiž tuto metodu doporučují pouze v případech, kdy není dobře určitelný bod návratu vlny T k izoelektrické linii.[15]

Kontroverzní je i metodika korekce intervalu QT k tepové frekvenci. Korekce podle Bazetta ($QTc = QT/RR^{1/2}$) je užívána nejčastěji (a to i k důležitým

regulačním rozhodnutím typu určení fyziologických rozmezí a k rizikové stratifikaci) a důvodem je jistě především jednoduchost. Existují však četné publikace dokladující neadekvátnost této metodiky, neboť interval QT korigovaný podle Bazetta je artificiálně prodloužen při frekvencích nad 60/min a naopak zkrácen při frekvencích pod 60/min.[16] Poměrně nedávno bylo prokázáno, že vztah QT/RR vykazuje významnou variabilitu mezi jednotlivými jedinci, zatímco naopak u konkrétního jedince zůstává i s větším časovým odstupem stabilní.[17] Z toho vyplývá, že nemůže existovat univerzální matematický vzorec, který by uspokojivě popisoval vztah QT/RR u všech jedinců; v podstatě se dá říci, že každý subjekt má „svou vlastní" rovnici.

STANOVENÍ DIAGNÓZY

Pro stanovení diagnózy LQTS je rozhodující klinický obraz, tedy zastoupení jednotlivých příznaků v rámci Schwartzova skóre. Jak již bylo uvedeno výše, až 1/3 nosičů patologických mutací však nemá ani prodloužený interval QT. K potrvzení klinické diagnózy LQTS jsou proto využívány různé testy navozující adrenergní stav, neboť adrenergní stimulace je významným spouštěcím faktorem arytmií. Jednou možností je podání izoprenalinu, výsledkem jsou ale relativně často bizarní vlny T s obtížně interpretovatelným koncem. Další nevýhodou této metody je nutnost parenterální aplikace léčiva. Existuje několik prací



Obr. 3 Polymorfní komorová tachykardie „torsades de pointes"

Charakteristickou, ba přímo patognomickou, arytmií LQTS je *polymorfní komorová tachykardie typu „torsades de pointes" (TdP) (obrázek 3)*. Je podkladem recidivujících synkop, a degeneruje-li ve fibrilaci komor, je pak i příčinou náhlé smrti. Bohužel, náhlá srdeční smrt nebo oběhová zástava je prvním příznakem u 13 % pacientů s LQTS.[12]

Schwartzovo skóre rozšiřuje klasické diagnostické známky o další položky. Jsou to jednak některé EKG fenomény:

- Tzv. *„alternans vlny T"*, tedy změna morfologie vlny T od komplexu ke komplexu (úkaz vzácný ale vysoce patognomický pro LQTS).
- *Dvojvrcholová vlna T* alespoň ve 3 svodech (opět *obrázek 2*) – studie s holterovským monitorováním nalezly dvouvrcholové vlny T u 80 % pacientů s LQTS2. Méně často je tento fenomén nacházen u LQTS1 a LQTS3, ale také bývá pozorován u 4 % zdravých jedinců v kontrolních skupinách.[18–20] Fyziologický mechanismus vzniku dvouvrcholové vlny T byl objasněn experimentálními studiemi. Komplexní tvar vlny T vzniká v důsledku napěťových gradientů mezi jednotlivými vrstvami myokardu.[21,22]
- Nízká tepová frekvence vzhledem k věku (především u dětí).

Dále je kladen důraz na *rodinnou anamnézu*, která je pozitivní až v 60 % případů a tedy každému pacientovi vyšetřovaného pro synkopu by měla být položena otázka na výskyt synkop či náhlé smrti v příbuzenstvu.

dokladujících, že přinejmenším u LQTS1 může test s izoprenalinem pomoci identifikovat latentní nosiče mutací.[23,24]

Naproti tomu ergometrie navozuje adrenergní stav přirozenou cestou a křivky EKG získané moderními ergometry mají velmi dobrou kvalitu, a tak umožňují i úspěšně hodnotit interval QT. Navíc se jedná o vyšetření neinvazivní. První práce využívající ergometrii v diagnostice LQTS se objevily na přelomu milénia,[25] včetně naší pilotní studie.[26] Ty ukázaly, že při zátěži dochází k dalšímu prodloužení intervalu QT, což napomáhá klinické diagnostice LQTS. Práce Takenaky a spol. a vzápětí i naše pilotní studie ukázaly, že fyzická zátěž může zvýraznit nebo i indukovat dvouvrcholovou morfologii vlny T,[25,27] nejspíše dalším zvýšením transmurální disperze repolarizace; nicméně tento fenomén zatím nebyl studován na experimentálním modelu LQTS. Zátěžová indukce dvouvrcholové morfologie vlny T přidává další bod ve Schwartzově skóre a tak přispívá významně ke klinické diagnostice LQTS.

ÚLOHA MOLEKULÁRNÍ GENETIKY V DIAGNOSTICE LQTS

Pro stanovení diagnózy LQTS je rozhodující klinický obraz. Mutační analýza je totiž časově náročná a její výsledky bývají k dispozici v ideálním případě týdny od odběru krevních vzorků. Vždy je nutno analyzovat všechny geny v celé jejich délce, neboť ve většině vyšetřených rodin bývá nalezena originální mutace. U téměř 50 % rodin není nalezena žádná mutace v genech, které byly dosud asociovány s LQTS. V těchto rodinách se předpokládá postižení dalších dosud

neznámých struktur, které by se mohly na procesech akčního potenciálu podílet.

Nález patologické mutace hraje významnou roli v potvrzení diagnózy, zejména u oligosymptomatických či asymptomatických příbuzných probanda. Naopak nenalezení mutace v dosud známých genech však diagnózu nevylučuje. Asociace klinického obrazu se zjištěnou mutací v genu pro některý iontový kanál není vždy jednoznačná. Jasným průkazem je v tomto případě pouze elektrofyziologická studie s mutovaným kanálem exprimovaným v heterologním buněčném systému. Méně spolehlivá je segregace konkrétní mutace s fenotypovým vyjádřením syndromu v dané rodině, na kterou jsme odkázáni v našich podmínkách.

V České republice je v současné době dostupná mutační analýza základního souboru 5 genů asociovaných s LQTS ve Fakultní nemocnici Brno (možno domluvit přes autora tohoto článku).

LQTS NA MOLEKULÁRNÍ ÚROVNI

LQTS typ 1 – chromosom 11p15.5, gen KCNQ1 (dříve KVLQT1), α-podjednotka IKs

Jak již bylo uvedeno výše, v roce 1991 prokázali Keating a spol. vazbu LQTS na krátké raménko 11. chromosomu u jedné velké rodiny v Utahu. Vlastní gen však dlouho odolával pokusům o identifikaci. Až v roce 1996 se ukázalo, že se jedná o mutace genu KCNQ1, který kóduje do té doby neznámý K^+ kanál.[28] Po určitých nejasnostech bylo pak zjištěno, že tvoří α podjednotku iontového kanálu nesoucího proud I_{Ks}, a to společně s proteinem minK (β podjednotka).[29,30] Výsledkem exprese defektních proteinů je vznik kanálů se změněnými vlastnostmi nebo i kanálů zcela nefunkčních (tzv. dominatně negativní efekt mutace). Dochází tak k oslabení výsledného repolarizačního proudu ve fázi 3 AP, k prodloužení trvání AP a tím i prodloužení intervalu QT.

LQT1 je zřejmě nejčastějším typem. Zahrnuje asi 50 % všech pacientů s LQTS, do dnešního dne bylo v genu KCNQ1 popsáno přes 100 různých mutací.[31] Pro tento typ je charakteristickým spouštěcím mechanismem arytmií adrenergní aktivace spojená s fyzickou i psychickou zátěží.[32] Vysoké riziko arytmií mají ti nositelé mutací genu KCNQ1, jejichž QT_c je delší než 0,5 s – kumulativní pravděpodobnost první příhody do 40 let věku je u takových jedinců 70%. Zajímavé je, že u mužů dochází k manifestaci dříve než u žen. Na druhé straně 36 % nosičů mutací genu KCNQ1 je asymptomatických, tedy nemají ani prodloužený interval QT a riziko arytmií je u nich nízké.[12]

V roce 1997 bylo dále zjištěno, že postižení tohoto genu je podkladem nejen Romanova a Wardova syndromu ale i syndromů Jervellova a Lange-Nielsenova. KCNQ1 je totiž exprimován i ve vnitřním uchu, kde se snad podílí na udržování endolymfatické homeostázy. V případě, že jsou postiženy obě alely genu a jedná se tedy o homozygotní jedince, manifestuje se i postižení sluchu.[33]

LQTS typ 2 – chromosom 7q35-q36, gen KCNH2 (dříve HERG), α-podjednotka I_{Kr}

Gen KCNH2 byl klonován v roce 1994 a vzápětí byl asociován s LQTS.[34] Kóduje α-podjednotku I_{Kr}. I zde byly popsány desítky různých mutací zodpovědné za asi 30–35 % případů LQTS. Kromě poruchy kinetiky iontového kanálu způsobují některé mutace KCNH2 poruchu intracelulárního transportu proteinů. Znamená to, že syntetizované podjednotky I_{Kr} se vůbec nedostanou do buněčné membrány.[35]

Zajímavým fenotypickým rysem LQTS2 je výskyt dvouvrcholových vln T (více viz níže). Spouštěcím momentem arytmií jsou kromě adrenergních situací také akustické podněty (typicky zvonění budíku nebo telefonu).[32] Rizikoví jsou opět jedinci, jejichž QT_c je delší než 0,5 s – kumulativní pravděpodobnost první příhody do 40 let věku je u takových jedinců 75%. Podíl asymptomatických nosičů je u LQTS2 nižší – 19 %.[12]

LQTS typ 3 – chromosom 3p21, gen SCN5A, α-podjednotka I_{Na}

Sodíkový kanál srdečních myocytů byl klonován v roce 1992 a jeho struktura se liší od většiny ostatních napěťově vrátkovaných iontových kanálů (homo- nebo heterotetramery). Tyto 4 podobné motivy jsou v případě I_{Na} obsaženy v jediném velkém proteinu,[8] takže produkt jediného genu je sám o sobě schopen vytvořit funkční kanál. S LQTS byl asociován již v roce 1995.[36] Mutace genu SCN5A se nacházejí asi u 15 % pacientů s LQTS, jejich počet je nižší (stejné mutace byly identifikovány ve více nepříbuzných rodinách). Odlišný je mechanismus prodloužení repolarizace, a tím i intervalu QT: dochází ke zvýšení a prodloužení trvání sodíkového proudu do buňky.[37]

Odlišné jsou i fenotypové projevy LQTS3: maligní arytmie se nejčastěji vyskytují v klidu nebo dokonce ve spánku.[32] Důležitým stratifikačním kritériem je zde pohlaví: při QT_c delším než 0,5 s je kumulativní pravděpodobnost první příhody do 40 let věku u mužů 80%, zatímco u žen 55%. Podíl asymptomatických nosičů je u LQTS3 10%.[12]

Zajímavé je, že mutace genu SCN5A jsou spjaty i s jinými arytmickými syndromy – Brugadovým syndromem (idiopatická fibrilace komor) a Lenegrovým syndromem (progresivní postižení převodního systému).[7]

LQTS 4 – chromosom 4q25-q27, gen ANK 2, cytoskeletární protein ankyrin B

Již v roce 1995 prokázala vazebná analýza vztah lokusu na 4. chromosomu k LQTS u jedné velké francouzské rodiny.[38] Až o 8 let později byl identifikován gen ANK2 kódující cytoskeletární protein ankyrin B.[39] Jak už napovídá jeho název, hraje tento protein důležitou roli v kotvení iontových kanálů do membrány – konkrétně Na/K-ATPázy a Na/Ca výměnníku – do membrány tubulů T sarkoplazmatického retikula srdečních myocytů. LQTS 4 se tak stal prvním typem, kde příčina není přímo v poruše iontového kanálu. Dosud bylo v genu ANK2 popsáno několik mutací, jejich vztah k LQTS ale není vždy zcela jasný, chybí totiž funkční studie. Rovněž není k dispozici dostatek údajů pro bližší charakteristiku tohoto typu LQTS.[40]

LQTS typ 5 – chromosom 21q22.1-p22.2, gen KCNE1, minK, β-podjednotka I_{Ks}

LQTS typ 6 – chromosom 21q22.1-p22.2, gen KCNE2, MiRP1 – β-podjednotka I_{Kr}

Tyto geny kódují vedlejší podjednotky iontových kanálů, které jsou nicméně nezbytné pro jejich správnou funkci. LQTS5 a LQTS6 jsou velmi vzácné, představují méně než 5 % všech případů, tedy i chybějí údaje k podrobnější specifikaci klinického obrazu. Vzhledem k postiženým kanálům se dá předpokládat, že fenotypické projevy LQTS5 odpovídají LQTS1, podobně LQTS6 odpovídá LQTS2.[7]

Andersen syndrom (LQTS typ 7), chromosom 17q23-24.2, gen KCNJ2, Kir 2.1 – podjednotka I_{K1}

Timothy syndrom (LQTS typ 8), chromosom 12p13.3, gen CACNA1C, α-podjednotka I_{CaL}

LQTS typ 9, chromosom 3p25, gen CAV3, kaveolární protein kaveolin 3

Dosud trvají diskuse, zda výše uvedené extrémně vzácné případy (často s komplexní neurologickou symptomatikou) zařazovat jako jeden z typů LQTS.[41–44]

LÉČBA LQTS

V léčbě LQTS se uplatňují

1. režimová opatření
2. betablokátory
3. implantabilní kardiovertery-defibrilátory (ICD)
4. kardiostimulace
5. levostranná sympatektomie

Režimová opatření

Bez ohledu na genotyp je třeba pacientům s klinickým obrazem LQTS doporučit některá režimová opatření.[45] Je vhodné vyvarovat se stimulů, které mohou působit jako spouštěcí faktor život ohrožující arytmie – náhlé hlasité zvuky (budík, telefon, zvonky). Nejbližší příbuzní by měli ovládat techniky laické resuscitace, v ideálním případě by domácnost měla být vybavena automatickým externím defibrilátorem (tyto přístroje jsou v současné době již komerčně dostupné). Personál školy či zaměstnání by měl být poučen, že každé omdlení je potenciálně velmi závažnou situací. Závodní sport je nevhodný, specificky je třeba zakázat skoky do vody, plavání jen v doprovodu jiné poučené osoby.

Betablokátory

Betablokátory (BB) jsou užívány v léčbě LQTS již od 70tých let. Cílem je potlačit adrenergní stimulaci, která se u většiny pacientů uplatňuje jako vyvolávající moment arytmií. Dvě dosud provedené studie (pouze observační, nikoli dvojitě slepé, natož pak placebem kontrolované, zahrnující 139 a 271 pacientů s geneticky potvrzenou diagnózou LQTS) ukazují až 80% redukci synkop a oběhových zástav po zahájení léčby BB, především u LQTS1 a LQTS2. Obě studie zpochybnily účinnost BB u LQTS3, může se však jednat o chybu malých čísel (do studií bylo zahrnuto 28, resp. 18 jedinců, s mutací genu SCN5A). Zajímavé je, že BB mají jen minimální účinek na délku QTc.[32,46]

Implantabilní kardiovertery-defibrilátory (ICD)

Implantabilní kardiovertery-defibrilátory (ICD) jsou nejúčinnější prevencí náhlé srdeční smrti. Bezpochyby jsou k implantaci ICD indikováni všichni jedinci, kteří byli resuscitováni pro maligní arytmie (bez ohledu na to, zda mají nebo nemají strukturální onemocnění srdce). Specificky u LQTS jsou pak za jasnou indikaci považovány rekurentní synkopy navzdory léčbě BB.[47] Vzhledem k tomu, že u LQTS je často nutná implantace již v dětském věku, pak vznikají problémy s velikostí ICD, opakovanými reimplantacemi z důvodu vybití baterií (baterie i v moderních přístrojích vydrží asi 4 roky) a v neposlední řadě problémy psychologického rázu.[45]

Kardiostimulace

Maligní arytmie u LQTS mohou být spuštěny bradykardií, která je samozřejmě potencovaná léčbou BB. Tomu má zabránit kardiostimulátor. Analýza souboru takto léčených pacientů však ukázala 24% rekurenci synkop či náhlých úmrtí v průběhu 6 let,[48] vhodnější by tedy u pacienta s LQTS a bradykardií byla implantace dvoudutinového ICD.

Levostranná sympatektomie

Tento postup je v léčbě LQTS užíván již od 70tých let a spočívá v odstranění dolní poloviny levostranného ganglion stellatum a dále druhého a třetího hrudního ganglia. Analýza zhruba 150 takto léčených pacientů ukázala významnou 80% redukci synkop či úmrtí.[49] Dnes je levostranná sympatektomie považována za doplňkovou léčbu pro pacienty, u kterých nemůžeme použít BB (např. z důvodu bronchiálního astmatu) nebo ICD (např. u velmi malých dětí).[45]

Konkrétní léčebné postupy[45]

1. Pacienti s LQTS po první prodělané příhodě: je indikováno nasazení BB. Pokud první příhodou byla resuscitovaná oběhová zástava nebo je QTc delší než 520 ms, pak je léčbou první volby ICD.
2. Asymptomatičtí jedinci (tzn. dosud neměli synkopu) s jasnou klinickou diagnózou LQTS (především s významným prodloužením QTc): je indikováno nasazení BB (pokud nejsou kontraindikace).
3. Pacienti s LQTS a recidivujícími synkopami navzdory léčbě LQTS: je indikována implantace ICD.
4. Zcela asymptomatičtí nosiči mutací v LQTS genech: přínos případné profylaktické léčby BB není jasný.

Sekundární (získaný) LQTS

Jen velmi krátce zmíním sekundární (získaný) LQTS, pro podrobnější informace odkazuji na velmi bohatou literaturu. Obraz LQTS může u dosud zdravého jedince vzniknout působením vnějších faktorů.[50,51] K prodloužení intervalu QT dochází nejčastěji vlivem celé řady kardiovaskulárních i nekardiovaskulárních léků *(tabulka III)* – blokátorů iontových kanálů myokardu (nejčastěji I_{Kr}), při elektrolytové dysbalanci (hypokalemie, hypomagnezemie a hypokalcemie způsobí prodloužení repolarizace ovlivněním vodivosti iontových kanálů),

Tabulka III
Léky prodlužující interval QT

Antiarytmika	ajmalin, amiodaron, bretylium, dofetilid, disopyramid, ibutilid, prokainamid, propafenon, chinidin, sotalol
Antibiotika, chemoterapeutika, antimykotika	amantadin, clarithromycin, chloroquin, cotrimoxazol, erythromycin, flukonazol, halofantrin, itrakonazol, ketokonazol, pentamidin, chinin, spiramycin, sparfloxacin
Antihistaminika	astemizol, loratadin, terfenadin
Psychofarmaka	amitryptilin, clomipramin, clozapin, chlorpromazin, citalopram, desipramin, doxepin, droperidol, fluphenazin, haloperidol, imipramin, lithium, maprotilin, mesoridazin, nortryptilin, pericyclin, pimozid, prochlorperazin, quetiapin, risperidon, sertindol, sultoprid, thioridazin, timiperon, trifluoperazon, zimeldin, ziprasidon
Různé	cisaprid, indapamid, ketanserin, probucol, sildenafil, vasopresin

Tabulka IV
Faktory zvyšující riziko proarytmie

Hraniční nebo i prodloužený interval QT před nasazením léku (QT_c nad 450 ms)
Ženské pohlaví
Vyšší věk
Lékové interakce (blokátory iontových kanálů, inhibitory cytochromu P450)
Hypokalemie, hypomagnezemie
Onemocnění srdce (nízká ejekční frakce, hypertrofie, kongenitální LQTS)

při lézích centrální nervové soustavy (mechanismus je nejasný). Plně vyjádřený sekundární LQTS se vyvíjí zpravidla až při kombinaci několika rizikových faktorů *(tabulka IV)*.

Prodloužení repolarizace při tzv. polékovém syndromu dlouhého intervalu QT by mohlo být způsobeno dosud latentními mutacemi genů pro tyto kanály, které se odkryjí až v zátěžové situaci, kterou může být právě podání rizikového léku. Dostupné údaje (včetně naší malé studie) však uvádějí, že mutace genů asociovaných s LQTS se nacházejí u 5–10 % osob s polékovými TdP.[52–55] Přestože všechny léky (kardiovaskulární i nekardiovaskulární) dosud asociované s prodloužením intervalu QT jsou účinné blokátory I_{Kr} kanálu, nelze fenomén proarytmie vysvětlit pouhým postižením na úrovni iontových membránových kanálů. Mechanismus polékového prodloužení intervalu QT je komplexní, obecně lze říci, že podkladem interindividuálních rozdílů v reakci na určité léčivo mohou být polymorfismy genů kódujících jak enzymy metabolismu léčiv, tak proteiny účastnící se transportu léků nebo cílové struktury pro léky.[56]

Další hereditární arytmické syndromy

Specifickým rysem LQTS je strukturálně normální nález na srdci. Absence strukturální patologie je typická i pro další hereditární arytmické syndromy, označované někdy též jako nemoci iontových kanálů – kanalopatie.

Syndrom krátkého intervalu QT (SQTS)

SQTS byl popsán v roce 2000.[57] Je charakterizován familiárním výskytem intervalu QT kratšího než 300 ms a častým výskytem maligních arytmií již v dětském věku. Dosud byly u jedinců s tímto syndromem nalezeny mutace genů KCNQ1, KCNH2 a KCNJ2.[58,59,60] Výsledkem je výrazné zkrácení repolarizace vedoucí ke zvýšení její transmurální disperze a tím ke zvýšení rizika maligních arytmií. Vzhledem k malému počtu pacientů s SQTS zatím nejsou k dispozici podrobnější údaje o klinickém průběhu tohoto onemocnění.

Brugadův syndrom

Tento syndrom, popsaný bratry Brugadovými,[61] je charakterizován EKG obrazem inkompletní blokády pravého raménka Tawarova ve svodech V1-3, na který navazují descendentní elevace úseku ST přecházející v negativní vlnu T. Klinicky se projevuje synkopami a náhlými úmrtími především u mužů mladších 50 let. Jak již bylo řečeno výše, na srdci není nacházena žádná strukturální patologie. Poměrně často se tento syndrom vyskytuje v jihovýchodní Asii, v místních jazycích se dokonce vyskytují jednoslovné termíny označující neočekávané úmrtí v noci ve spánku („*bangungut*" v Thajsku nebo „*pokkuri*" v Japonsku).[62] Asi u 20 % pacientů s tímto syndromem se nacházejí mutace genu SCN5A (kódující α-podjednotku sodíkového kanálu). Mutace stejného genu mohou vyvolat i obraz LQTS3 (viz výše).

Katecholaminergní polymorfní komorová tachykardie (CPVT)

Jedná se o vzácnou autosomálně dominantně dědičnou arytmii, která se manifestuje často již v dětském věku a způsobí náhlou srdeční smrt až u 30 % nemocných do věku 30 let.[63,64] Asi u 40 % postižených jsou nacházeny mutace v genu pro ryanodinový receptor (RyR), což je vápníkový kanál sarkoplazmatického retikula kardiomyocytů. Hraje zásadní roli v regulaci intracelulárních přesunů vápníku. Ve sporadických případech jsou nacházeny mutace v genu pro calsequestrin. Ten je součástí makromolekulárního bílkovinného komplexu ve vazbě na RyR.[65]

ZÁVĚR

I když bylo v poslední dekádě dosaženo obrovského pokroku v chápání LQTS, zůstává řada nevyřešených problémů. Původní relativně jednoduchý koncept LQTS jako nemoci iontových kanálů naboural objev mutací cytoskeletárního proteinu ankyrinu u této choroby. Téměř polovina jedinců s klinickým obrazem LQTS navíc nemá detekovatelnou mutaci v žádném z genů se známým vztahem k LQTS. U nich mohou být v budoucnu objeveny zcela nové patofyziologické mechanismy založené například na poruchách regulačních proteinů.

Odhadovaná prevalence LQTS je asi 1 : 10 000. Nedávno však Schwartz a spol. provedli mutační screening všech známých genů LQTS u 130 probandů LQTS, u kterých byla již dříve nalezena mutace v jednom z uvedených genů. V tomto souboru byla překvapivě u 6 jedinců (4,6 %) nalezena další nezávislá mutace v genech LQTS. Takoví jedinci jsou označováni jako složení heterozygoti.[66] V případě prevalence 1 : 10 000 je však pravděpodobnost tohoto jevu 1 : 100 000 000. Tyto údaje ukazují, že výskyt polymorfismů genů pro iontové kanály bude zřejmě mnohem vyšší než se dosud domníváme.

Nedávno byly publikovány výsledky dvou geneticky zaměřených populačních studií,[67,68] sledujících rizikové faktory náhlé srdeční smrti. Byla v nich provedena multivariační analýza nejen konvenčních rizikových faktorů pro ischemickou chorobu srdeční (faktory biologické, dietetické a faktory prostředí). Ukázalo se, že pozitivní rodinná anamnéza náhlé srdeční smrti je silným nezávislým rizikovým faktorem náhlé srdeční smrti pro potomstvo. Anamnéza náhlého úmrtí u rodiče zvyšuje 1,8krát riziko náhlého úmrtí pro potomka. V případě náhlého úmrtí obou rodičů pak relativní riziko pro potomstvo dosahuje překvapivě vysoké hodnoty 9,4. I když vezmeme v úvahu metodologická úskalí daného problému, podávají tyto studie přesvědčivý důkaz o familiárním výskytu náhlé srdeční smrti i v běžné populaci.[69] Při vědomí familiárně společně sdílených vnějších rizikových faktorů je zřejmá také existence geneticky podmíněných odchylek různých fyziologických procesů, které zvyšují riziko náhlé srdeční smrti. Mutace jednotlivých genů zde pravděpodobně hrají jen malou roli, etiologie je jistě polygenní. Znamená to, že se mohou uplatňovat i tzv. běžné polymorfismy DNA (tedy odchylky v sekvenci DNA, které se vyskytují v různém procentu v populaci a jsou považovány za varianty normy).

Z uvedených údajů vyplývá i klinický význam studia LQTS pro celou populaci. Klinicky zdraví jedinci (s normální délkou intervalu QT) totiž mohou mít latentní redukci repolarizačních proudů, „sníženou repolarizační rezervu“, neboť jsou nosiči polymorfismů nebo mutací v genech pro iontové kanály.[70] Ty se mohou projevit až v zátěžových podmínkách, například při ischemii myokardu nebo po podání jakéhokoli léku blokujícího některý kanál důležitý pro repolarizaci. V blízké budoucnosti mohou být takovéto změny v sekvenci DNA rozpoznány jako dědičné molekulární rizikové faktory kardiovaskulárních chorob a dát vznik zcela nové generaci jak diagnostických, tak terapeutických metod.[71]

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2.1.1 Poznámky k patofyziologii LQTS

Patofyziologie LQTS byla podrobně probrána ve výše vloženém článku. Zde bude uvedeno několik poznámek k homo- a heterozygotní formě LQTS.

Jak již bylo uvedeno, běžnou formou LQTS je Romano-Ward syndrom (RWS). Pokud jsou klasické příznaky vzácně doplněny ještě hluchotou, hovoříme o Jervell a Lange-Nielsen syndromu (JLNS). Po odkrytí genetické podstaty LQTS bylo poměrně brzy zjištěno, že obě varianty mohou být způsobeny mutacemi ve stejných genech, pacienti s RWS jsou heterozygoti, u pacientů s JLNS byly mutace genů kódující podjednotky iontového kanálu nesoucího proud I_{Ks} nalezeny v homozygotní formě. Tento kanál je totiž přítomen i ve vnitřním uchu a jsou-li postiženy obě alely, je kromě postižení srdce přítomno i postižení sluchu.

Této problematice jsme se věnovali ve dvou následně vložených publikacích.

<u>Novotný T</u>, Kadlecová J, Janoušek J, Gaillyová R, Bittnerová A, Florianová A, Šišáková M, Toman O, Chroust K, Papoušek I, Špinar J. The homozygous KCNQ1 gene mutation associated with recessive Romano-Ward syndrome. Pacing Clin Electrophysiol 2006;29:1013-1015.

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(publikovaný popis případu - kvantitativní podíl uchazeče 30 % - koncept, koordinace práce zúčastněných pracovišť, klinické vyšetřování pacientů, analýza dat, text publikace)

Obsahem článku je podrobná klinická i genetická charakteristika rodiny s výskytem LQTS pouze u jednoho z dětí. Jedná se první popis homozygotní mutace genu *KCNQ1* u potomstva nepříbuzenského sňatku, což bylo v té době považováno za vysoce nepravděpodobné. Tento nález podporoval posléze prokázanou hypotézu o násobně vyšším výskytu mutací genů pro iontové kanály myokardu. Funkční efekt nalezené mutace je zřejmě omezený, neboť u heterozygotních rodičů nevedla k obrazu LQTS, a v homozygotní formě se sice objevily příznaky LQTS, avšak bez hluchoty. U rodičů tedy tuto mutaci můžeme označit za latentní. Obě výše uvedená fakta jsou základem hypotézy o významné roli variant těchto genů v patofyziologii náhlé srdeční smrti v běžné populaci.

CASE REPORTS

The Homozygous *KCNQ1* Gene Mutation Associated with Recessive Romano–Ward Syndrome

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In a 7-year-old boy with normal hearing suffering from repeated syncope an extremely prolonged QT_c interval (up to 700 ms) was found. The mother was completely asymptomatic and the father had an intermittently borderline QT_c interval (maximum 470 ms) but no symptoms. In the proband a mutation analysis of KCNQ1 gene revealed a homozygous 1893insC mutation. The parents were heterozygous for this mutation. There was no consanguineous marriage in the family. The clinical relevance of these findings is that apparently normal individuals may have a latent reduction of repolarizing currents, a "reduced repolarization reserve," because they are carriers of latent ion channel genes mutations. (PACE 2006; 29:1013–1015)

ion channel, KCNQ1, long QT syndrome, sudden death

Mutations in the *KCNQ1* gene (GenBank accession no. AF000571) encoding the α subunit of the *KCNQ1* channel cause both the recessive Jervell and Lange–Nielsen syndrome (homozygous mutation) and more frequent dominant Romano–Ward syndrome (heterozygous mutation). The Jervell and Lange–Nielsen syndrome is also associated with a congenital bilateral deafness.[1,2] Here we present a rare evidence for Romano–Ward syndrome caused by homozygous *KCNQ1* mutation.

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Case Report

A 7-year-old boy was referred to a pediatric cardiology unit because of very frequent syncopal events during physical or emotional stress, while having been treated for several years for suspected

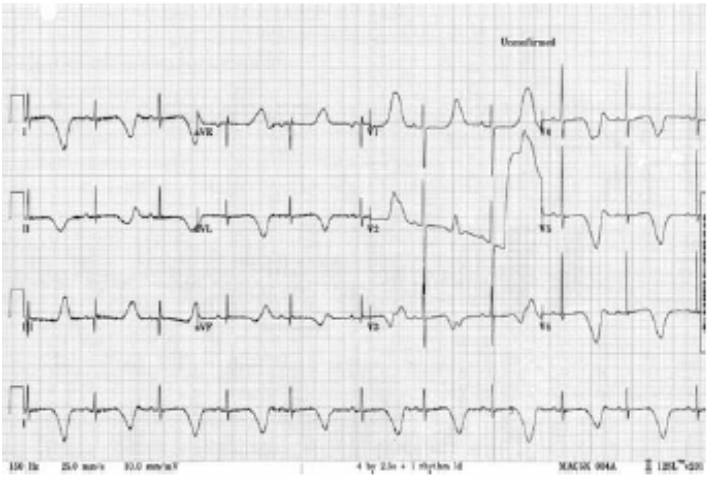

Figure 1. *ECG recording of the proband—homozygous carrier of 1893insC mutation of* KCNQ1 *gene. RR interval 1000 ms, uncorrected QT interval ranging from 600 to 640 ms.*

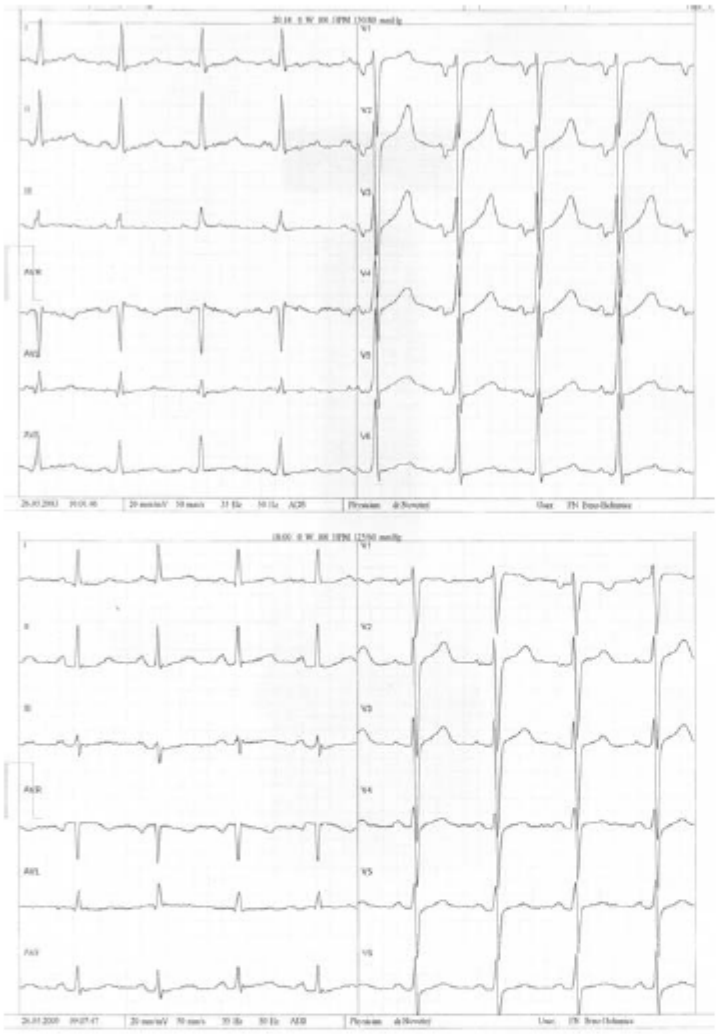

Figure 2. *ECG recordings of the proband's parents 6 minutes after cessation of exercise. Upper ECG—father-RR interval 640 ms, QT interval 340 ms, QT_c (Bazett) 420 ms. Lower ECG—mother-RR interval 600 ms, QT interval 300 ms, QT_c (Bazett) 440 ms.*

epilepsy. The general medical evaluation was unremarkable but on resting electrocardiogram (ECG) an extremely prolonged QT_c interval of 700 ms was present. During bicycle exercise QT_c intervals ranging from 490 ms to 600 ms were recorded (Fig. 1). The boy was treated with an ICD and the beta-blocking agent atenolol. Audiometry was performed and no hearing disorder was found. The mother of the proband was completely asymptomatic. During bicycle ergometry QT_c values did not exceed physiological values. The father had no history of syncope. A slightly prolonged QT_c interval (470 ms) was, however, recorded immediately after cessation of exercise. In the other phases of the stress test, QT_c values did not exceed physiological limits (Fig. 2). All ECGs were obtained with a sweep of 50 mm/s and voltage of 20 mm/mV. QT intervals were measured manually by two blinded investigators and corrected to the heart rate using the Bazett formula.

Informed consent was obtained from all the individuals and peripheral blood samples were taken.[3] All exons of *KCNQ1* gene and their flanking intronic sequences were amplified by multiplex polymerase chain reaction (PCR) with primers described by Larsen et al.[4] Multiplex single-stranded conformation polymorphism (SSCP) and DNA sequence analyses were used to screen for *KCNQ1* gene mutations (ABI 310 sequencer, Applied Biosystems, CA, USA).

In the proband, mutation analysis of *KCNQ1* gene found a homozygous 1-bp insertion of cytosin at a position of 1893 (1893insC) that leads to premature stop codon. This mutation has so far been related to a long QT syndrome, but only in a heterozygous state.[5] The parents of the proband were heterozygous for the mutation 1893insC. They were not aware of any consanguineous marriage in the family.

Discussion

According to our knowledge only two other cases were described previously. The first one was a consanguineous family in which a *KCNQ1* gene mutation was associated with long QT syndrome phenotype only in the homozygous proband. Both parents were heterozygous carriers of the mutation and had a normal phenotype.[6] In the other family, the proband and his brother were suffering from a severe Romano–Ward syndrome associated with compound heterozygosity for two mutations in the *KCNQ1* gene. The mutations were found to segregate as heterozygotes in the maternal and the paternal lineage, respectively. None of the heterozygotes exhibited clinical LQTS.[7]

In the above-described Czech family, a 1893insC mutation of *KCNQ1* gene was found. The same mutation has been described previously in heterozygous patient with fully symptomatic Romano-Ward syndrome.[5] It means that the same mutation can behave either dominantly or recessively while expressed in different genetic background. For this particular mutation no cellular expression studies are available. There are some data suggesting that KCNQ1 C-terminal mutations impair subunit assembly or trafficking, usually not exhibiting the dominant negative effect. This is the possible explanation for marked differences in phenotypic expression in homo- and heterozygous carriers.[8,9]

In all three cases both parents were asymptomatic despite being mutation carriers. It supports a hypothesis that the spectrum of the genetic abnormalities in ion channel genes may include "mild" mutations and it raises the issue of paragenetic control or other genetic and environmental modulators.

The indicated prevalence of long QT syndrome is about 1/10,000. But in that case the probability of a single individual having two mutations would be 1 in 100 million. Nevertheless, recently Schwartz et al. performed molecular screening for all known long QT syndrome genes in 130 LQTS probands. Six individuals (4.6%) were identified as compound heterozygous carriers of independent mutations in long QT syndrome genes.[10] These data show that the prevalence of ion channel gene polymorphisms must be much higher than hypothesized thus far.

The clinical relevance of these findings is that apparently normal individuals (with normal QT interval) may have a latent reduction of repolarizing currents, a "reduced repolarization reserve," because they are carriers of ion channel gene mutations or polymorphisms.[11] They may become symptomatic under stress conditions; for example, under myocardial ischemia or challenge by drugs blocking any ion channel that is important for repolarization. In the near future such DNA sequence changes could be recognized as inheritable molecular risks of cardiovascular diseases bringing up a completely new generation of both diagnostic and therapeutic methods.[12–14]

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Obsahem článku je podrobná klinická i genetická charakteristika rodiny s výskytem LQTS. Rodiče jsou vzdálení příbuzní a oba jsou nositeli identické mutace genu *KCNQ1*, jsou asymptomatičtí a prodloužený interval QT byl u nich přítomen pouze při zátěžovém testu. Obě jejich dcery jsou homozygoty pro tuto mutaci a LQTS je u nich naopak výrazně vyjádřen, avšak pouze jedna z nich má audiometricky prokázanou poruchu sluchu. Identická homozygotní mutace tedy v jednom případě vedla k projevům Romano – Ward syndromu a ve druhém Jervell a Lange-Nielsen syndromu. Význam tohoto nálezu pro chápání patofyziologie LQTS je podrobně probrán v diskuzi vloženého článku.

A new homozygous mutation of the *KCNQ1* gene associated with both Romano-Ward and incomplete Jervell Lange-Nielsen syndromes in two sisters

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Background

The mutations in the *KCNQ1* gene (GenBank accession no. AF000571) encoding the α subunit of the KCNQ1 channel can cause 2 different diseases: Romano-Ward syndrome (RWS), traditionally described as a combination of repeated syncope episodes and a prolonged QT interval, and the less frequent Jervell and Lange-Nielsen syndrome (JLNS), also associated (except for the abovementioned symptoms) with congenital bilateral deafness.[1] The first one is usually associated with the heterozygous gene mutation, the latter one with the homozygous mutation. The disease prevalence is estimated at close to 1 in 2,500 live births, and JLNS has been reported to affect about 3 in 1 million individuals, which represents less than 1% of all long-QT syndrome (LQTS) patients.[2]

Case report

A 19-year-old woman suffered from a sudden syncope at her new job. After the ambulance arrived, the syncope repeated and a polymorphic ventricular tachycardia torsades de pointes was identified on the electrocardiogram (ECG). During the second syncope, it terminated spontaneously. The patient was admitted to the local hospital. A prolongation of the QT interval was identified, and the patient was referred to the specialized cardiology center for further examination and treatment. Extremely prolonged QTc (Bazett formula) of 620 ms was recorded on admission (Figure 1A).

KEYWORDS KCNQ1; Jervel Lange-Nielsen syndrome; Romano-Ward syndrome

ABBREVIATIONS **ECG** – electrocardiogram; **ICD** – implantable cardioverter-defibrillator; **JLNS** – Jervel Lange-Nielsen syndrome; **RWS** – Romano-Ward syndrome (Heart Rhythm 2010;7:531–533)

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The detailed patient history revealed repeated syncope episodes since the age of 2 years, usually 1 to 5 times per year, lasting 30 to 60 seconds, and provoked by stress. Every time the emergency personnel arrived, the patient was already conscious. All syncope episodes were accompanied by cyanosis. During the admission interview, we found that the patient had a 21-year-old sister with almost the same symptoms. She had suffered from syncope episodes since the age of 4 years old, and the frequency was much higher. She sometimes became unconscious, even several times per day during her adolescence; during the year of examination, the frequency was several times per year. QTc of 670 ms was found on the ECG (Figure 2), and the patient also was admitted to our hospital. The general medical examination was unremarkable in both sisters; no abnormalities were found in biochemical or hematological blood test results. Echocardiography was performed, showing excellent systolic function with a left ventricular ejection fraction of 72% in the younger sister and 81% in the older one. No morphologic abnormality was discovered. Both patients underwent a bicycle stress test, which revealed a prolonged QTc with further prolongation during exercise and resting phase (700 to 750 ms in the younger sister and 650 to 680 ms in the older one). Bizarre negative T waves developed during the exercise (Figure 1B), and then notched T waves developed during the resting phase (Figure 1C) in the younger patient.

The audiometry revealed a subclinical hearing impairment in the younger sister, defined as a bilateral symmetric sensorineural hypacusis with the basocochlear loss of 60 dB. Thus, a diagnosis of incomplete JLNS syndrome was established in this case. In the older sister, the audiometry finding was physiological. Both sisters were primarily treated with an implantable cardioverter-defibrillator (ICD) because of the extremely prolonged QT interval and repeated aborted circulatory arrests. The beta-blocking agent betaxolol also was administered.

We also examined the patients' parents and found a consanguineous marriage in the family; the grandparents

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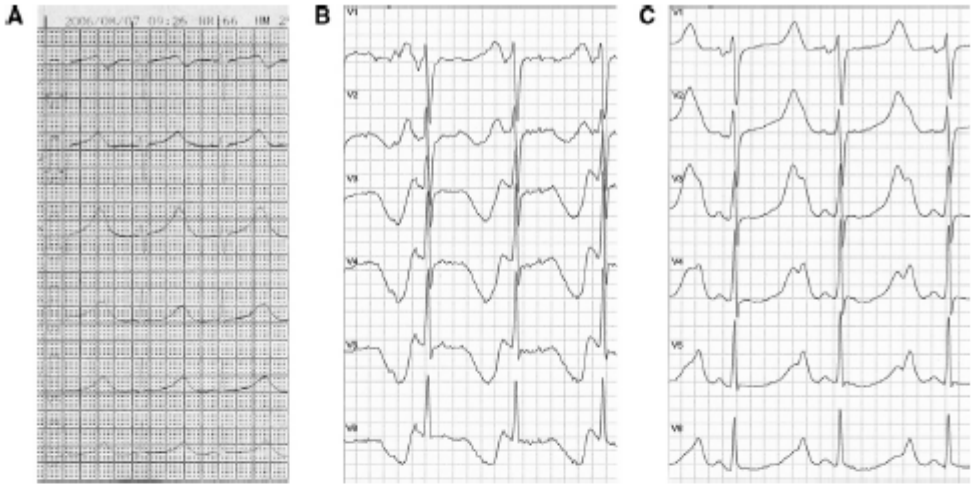

Figure 1 ECGs from the younger sister (chest leads). **A.** ECG of the younger sister recorded at admission to the cardiology center (RR interval 920 ms, uncorrected QT interval 600 ms). **B.** Bizarre negative T waves on ECG during the bicycle stress test. **C.** Notched T waves on ECG at the restitution phase of the stress test, typical for carriers of LQTS. ECG = electrocardiogram.

were cousins (Figure 3). The parents were asymptomatic, had no history of syncope, and the resting QTc was normal. During the stress test, there was a prolongation of QTc in both parents (470 ms in the father, 500 ms in the mother); the diagnosis of LQTS also was established in parents.

All of the individuals gave their informed consent, and peripheral blood samples were taken. Mutation analysis of LQTS-related genes was performed using the multiplex polymerase chain reaction, single-strand conformation polymorphism and DNA sequencing analyses.[3] Mutation R190L in the region between transmembrane segments S2-S3 of the *KCNQ1* gene was identified in all members of the family. The children were homozygous for this mutation; the parents were heterozygous. The mutation has not been described yet.

During the next 3 years, the younger sister had 2 episodes of syncope; both were recorded by the ICD as a polymorphic ventricular tachycardia with a frequency of 260 beats/min and terminated by the ICD shock therapy. The older sister has not had a syncope episode since the implantation.

Discussion

In this family, we have found a unique combination of LQTS symptoms in particular family members. The parents were heterozygous for the R190L mutation of the *KCNQ1* gene. They were completely asymptomatic, with normal resting QT intervals. There was no history of sudden cardiac death in the older generations. However, during the stress test, we have proved a pathological QTc in both parents. In

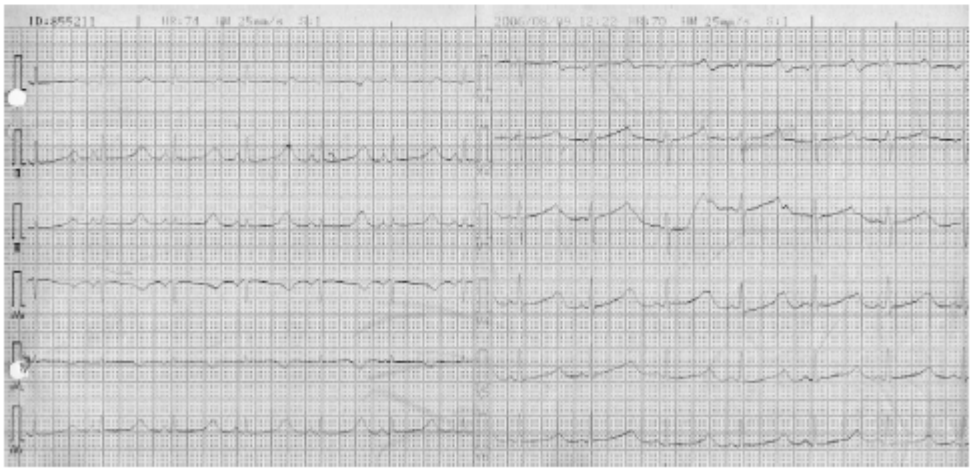

Figure 2 Admission ECG of the older sister. ECG recorded at admission to the cardiology center. RR interval 880 ms, uncorrected QT interval 600 ms. ECG = electrocardiogram.

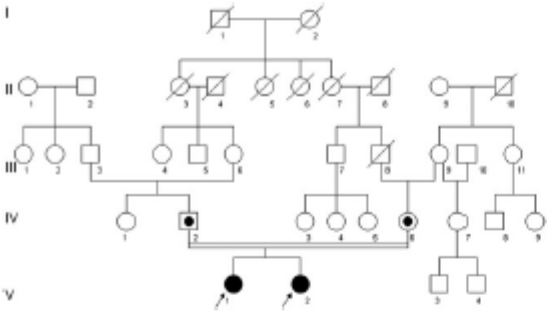

Figure 3 Pedigree of the family. V/1,V/2: Probands, genotype KCNQ1: (R190L)+(R190L). IV/2: Father, genotype KCNQ1: (R190L)+(−). IV/6: Mother, genotype KCNQ1: (R190L)+(−). The probands' grandparents were cousins.

addition, there were the homozygous children with very frequent syncope episodes and extreme QTc prolongation. The homozygous LQTS individuals usually present as JLNS with hearing impairment. However, in this case report, we have described the manifestation of both the RWS and the incomplete JLNS in sisters with a homozygous mutation in the *KCNQ1* gene. The manifestation of the RWS in homozygous patients is very rare.[4-6] As far as we know, this is the first report of a manifestation of both syndromes in siblings with the same mutation, although JLNS is manifested with incomplete hearing impairment.

JLNS is by definition associated with bilateral deafness. Thus, there is a possibility that the hearing impairment in the younger sister may have been caused by other factors (such as exposure to noise), but there is no evidence of such factors in the medical history of the patient. The diagnosis of incomplete JLNS is supported by the following features: 1) The bilateral sensorineural hearing impairment is symmetrical. 2) We observed a difference in the syncope frequency in both women. The one with the hearing impairment suffers from syncope episodes due to malignant arrhythmias despite beta-blocker therapy, and during her stress test, a more pronounced QT interval prolongation was present. More severe clinical manifestation of JLNS compared to RWS was reported repeatedly in the literature.[7]

It is not clear why the same homozygous mutation causes JLNS in one sibling and RWS in the other one. The KCNQ1 channel is expressed not only in the heart but also in the inner ear. There it plays an important role in the generation of the endocochlear potential necessary for normal hearing.[8-10] Most JLNS mutations of the *KCNQ1* gene are complex mutations, likely to interfere with subunit assembly and causing complete abolishment of ion channel function.[11] In a recent study, it has been shown that approximately 10% of the normal KCNQ1 current is able to maintain hearing function in homozygous carriers as well.[12] The functional effect of the R190L mutation discovered in this family is not known. Previously, a R190Q mutation was described in the same position.[13] In that study, the mutation led to a nonfunctional channel. This cannot be the case in this family because neither of the homozygous carriers was deaf. The diverse effect of a different mutation in the same position is probably because glutamine (Q) and leucine (L) residues are quite different in structure and chemical features. In the case of our R190L mutation, we can speculate that the proportion of functional channels is somewhere close to the abovementioned 10%. This means that in the older sister this amount is sufficient for normal hearing, but in the younger one some additional yet-unknown factors led to hearing impairment. This raises the issue of paragenetic control or other genetic and environmental modulators. The uncertainty of understanding these processes is often expressed as "the different genetic background of an individual." Despite great hopes expressed after the sequencing of the human genome several years ago, our understanding of the genetic background of pathophysiological processes is still quite poor.

The parents with the normal resting QTc in our case are an example of the latent carriers of a possibly malignant disease. In some LQTS families, only 25% penetrance of fully expressed disease was observed. Other carriers of pathologic mutations remained completely asymptomatic, that is, they did not present even a prolonged QT interval.[14] That is why we consider the stress test very important for the diagnostics of LQTS. The presented case report further extends our view of genotype–phenotype relationships in LQTS, which are anything else but simple. To elucidate them we will have to move to a higher level of understanding the regulatory processes of the human genome.

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2.1.2. Interval QT a jeho zátěžově závislé chování – komentář k současným poznatkům

Závislost intervalu QT na tepové (závislost QT/RR) je známa více než 100 let (9, 10) a byla předmětem celé řady studií (11). Zjednodušeně je možné říci, že interval QT se postupně zkracuje se zvyšující se tepovou frekvencí. Elektrokardiografická data nejrůznějšího původu, kvality a rozsahu byla zkoumána převážně s cílem formulovat obecně platný popis závislosti QT/RR a také získat široce využitelný matematický vzorec pro korekci intervalu QT k tepové frekvenci (QTc –interval QT korigovaný k tepové frekvenci). Žádný z těchto pokusů však nebyl opravdu úspěšný (12) a relativně nedávno bylo potvrzeno, že závislost QT/RR je velmi stálá u konkrétního jedince, nicméně interindividuálně vykazuje vysokou variabilitu (13, 14). Tedy žádná obecně platná korekční formule neexistuje a každý jedinec „má svůj vlastní korekční vzorec“. Tento fakt výrazně ztěžuje rozlišení fyziologického a patologického chování intervalu QT, byť je přesvědčivě prokázáno, že jak jeho zkrácení, tak i prodloužení je spjato se zvýšeným rizikem arytmií.

Byla navržena celá řada numerických charakteristik, jejichž cílem je jednak pokrok v chápání fyziologie repolarizačního procesu a také rozlišení mezi normální a abnormální repolarizací v klinicky dobře definovaných populacích. Jedná se o nejrůznější matematické metody od jednoduchých kalkulací plochy pod vlnou T (15) až po trojrozměrné rekonstrukce vektorokardiografických smyček vln T a jejich kvantifikace (s nutností využití algoritmů dekompozice na singulární hodnoty a rekonstrukce ve vícerozměrných algebraických prostorech) (16, 17).

Nedávné pokroky v digitální elektrokardiografii a umožňují provádění dlouhodobých EKG záznamů a jejich měření s vysokou přesností. Ve srovnání s pokrokem v aplikované elektrokardiografii je však podrobné chápání repolarizační elektrofyziologie mnohem méně pokročilé. Vztahy mezi různými charakteristikami EKG záznamu jsou převážně neznámé a není ani jasné, zda se různé deskriptory vztahují k různým aspektům stejného fyziologického procesu nebo odrážejí různé fyziologické procesy. K vysvětlení těchto nejasností bude třeba provést celou řadu cílených fyziologických studií nejrůznějších repolarizačních charakteristik za různých podmínek.

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Práce se věnuje fenoménu QT/RR hystereze – t. j. zpožďování zkracování intervalu QT za zkracováním intervalu RR. Prokazuje individuální specificitu a interindividuální variabilitu tohoto parametru a jeho nezávislost na statickém vztahu QT/RR.

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Subject-specific profiles of QT/RR hysteresis

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[1]*Saint Paul's Cardiac Electrophysiology, London, and* [2]*Saint George's, University of London, London, United Kingdom;* [3]*University Hospital Brno, Masaryk University, Brno, Czech Republic; and* [4]*Medizinische Klinik, Technische Universität München, Munich, Germany*

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Malik M, Hnatkova K, Novotny T, Schmidt G. Subject-specific profiles of QT/RR hysteresis. *Am J Physiol Heart Circ Physiol* 295: H2356–H2363, 2008. First published October 10, 2008; doi:10.1152/ajpheart.00625.2008.—The time lag of the QT interval adaptation to heart rate changes (QT/RR hysteresis) was studied in 40 healthy subjects (18 females; mean age, 30.4 ± 8.1 yr) with 3 separate daytime (>13 h) 12-lead electrocardiograms (ECG) in each subject. In each recording, 330 individual 10-s ECG segments were measured, including 100 segments preceded by 2 min of heart rate varying greater than ±2 beats/min. Other segments were preceded by a stable heart rate. In segments preceded by variable rate, QT/RR hysteresis was characterized by λ parameters of the exponential decay models. The intrasubject SDs of λ values were compared with the intersubject SD of the individual means. The λ values were also correlated to individually optimized parameters of heart rate correction. Intrasubject SDs of λ were substantially smaller than the population SD of individual means (0.390 ± 0.197 vs. 0.711, $P < 0.0001$). The λ values were unrelated to the QT/RR correction parameters. When compared with the corrected QT (QTc) for averaged RR intervals in 10-s ECGs and with the averaged RR intervals in 2-min history, QTc for QT/RR hysteresis led to a substantially smaller SD of QTc values (11.4 ± 2.00, 6.33 ± 1.31, and 4.66 ± 0.85 ms, respectively, $P < 0.0001$). Thus the speed with which the QT interval adapts to heart rate changes is highly individual with intrasubject stability and intersubject variability. QT/RR hysteresis is independent of the static QT/RR relationship and should be considered as a separate physiological process. The combination of individual heart rate correction with individual hysteresis correction of the QT interval is likely to lead to substantial improvements of cardiac repolarization studies.

QT adaptation; individual QT correction; electrocardiogram measurement; corrected QT variability

HEART RATE DEPENDENCY of the electrocardiographic QT interval has been the subject of numerous studies. Electrocardiographic data sets of variable sizes, sources, and quality have been used to investigate the relationship of the QT interval to heart rate (3, 12, 14, 26, 28), most frequently with the aim of finding a universally applicable heart rate correction formula. On the contrary, the adaptation of QT interval duration to rapid rate changes, which is the lag with which QT interval duration responds to heart rate changes, has been studied much less frequently. Indeed, in numerous studies that investigated the relationship between QT interval and heart rate in short-term (such as 10 s) electrocardiograms (ECG), the QT adaptation to heart rate changes has been, as a rule, impossible since no heart rate history preceding the measured ECGs was available.

The time scale of the adaptation of QT interval to heart rate changes, most frequently termed the QT/RR hysteresis, has been previously approximated in investigations with constant pacing rates. Such experiments measured both the action potential durations (11) and QT intervals in surface ECGs (17). Studies of QT/RR hysteresis in long-term ECG recordings without pacing provocation appeared only recently, made possible by the electronic capture and analysis of substantial ECG data. Differences in the QT/RR hysteresis profile were reported to stratify the risk in survivors of myocardial infarction (25, 29). It has also been reported that the correction of the QT interval for both heart rate and QT/RR hysteresis leads to more stable corrected QT (QTc) data with possible implications for clinical studies of repolarization changes (20). Proposals have also been made that the graphic display of the QT/RR hysteresis might be helpful in the detection of drug-induced QT interval changes (10).

Still, there is little knowledge on the basic physiology of QT/RR hysteresis, and detailed investigations in healthy subjects are lacking. With this in mind, this study investigated QT/RR hysteresis in a population of healthy subjects for whom repeated high-quality, long-term, 12-lead ECG recordings were available. The study aimed at studying whether QT/RR hysteresis can be consistently measured in long-term 12-lead ECGs, whether the hysteresis profiles differ among healthy subjects, and whether there is any relationship between the static QT/RR relationship and the dynamic QT/RR hysteresis.

METHODS

Population. The data of the study originated from clinical investigation CARISEPY 1025 sponsored by Johnson & Johnson Pharmaceutical Research and Development (Titusville, NJ). The study included repeated, long-term, 12-lead ECG recordings obtained during daytime hours. For the purposes of this investigation, the data were available in 40 healthy subjects (18 females; mean age, 30.4 ± 8.1 yr; range, 19 to 48 yr; interquartile range, 24 to 36 yr). Female subjects were marginally older (32.3 ± 19.4 yr) than male subjects (28.9 ± 6.7 yr), but the difference was not statistically significant. All subjects had a normal physical examination and a normal resting ECG at the study onset. During the ECG recordings of this investigation, the subjects were not on any medication, including hormonal contraceptives or hormone replacement therapy, homoeopathic or herbal remedies, and dietary supplements. They also refrained from smoking or digesting caffeine or alcohol. Also, during the actual ECG recordings, the subjects were not permitted to sleep. The study was conducted at the clinical facility of PPD Development (Austin, TX) and was approved by the Ethics Committee of the facility. All subjects gave informed, written consent.

ECG recordings. This investigation used ECG recordings obtained during the baseline of three phases of clinical study CARISEPY 1025 when the subjects were not exposed to any investigational drug. Thus,

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for the purposes of this investigation, each subject underwent three long-term, digital 12-lead ECG recordings that were initiated between 7 AM and 8:00 AM and lasted ≥13 h. Periods of 2 to 3 wk separated individual recordings in each subject, but the recordings were obtained in the same place and under the same environmental conditions. The recordings were obtained with SEER MC digital portable electrocardiographs (GE Healthcare, Milwaukee, WI) with the electrodes placed in Mason-Likar positions. The electrode placements were marked on the skin of each subject, and the electrode positions were carefully reproduced from recording to recording.

Selection of ECG data for QT interval measurements. Short-term, 10-s ECG segments were selected in each long-term ECG recording for an accurate QT interval measurement with the aim of characterizing *1*) QT/RR profile in which the QT interval durations have been already adapted to heart rate and *2*) QT interval adaptation to heart rate due to QT/RR hysteresis. Specifically, the following three separate steps of short-term ECG extractions were used.

During each recording period, the subjects were placed 26 times into standardized positions, mainly supine but also unsupported sitting and supporting standing. Each of these stable positions defined a 5-min window during which up to five 10-s ECG segments were selected from the long-term recording, all preceded by ≥90 s of stable heart rate with fluctuations less than ±2 beats/min with the heart rate calculated in 10-s segments. The selected ECG segments were not adjacent, and all of the ECG segments preceded by stable heart rate had the lowest noise content (1). The noise was characterized by a square root of the product of the SD noise and the root mean square of successive difference noise (1). This procedure defined up to 130 segments in each long-term recording.

In addition, all 10-s ECGs were found in each recording that were preceded by >120 s of stable heart rate (variability, less than ±2 beats/min). These were sorted according to the preceding stable heart rate and divided, in each long-term recording, into 100 bins as equidistant as possible in the underlying heart rate. In each bin, the ECG with the lowest noise content (1) was selected.

Furthermore, all 10-s ECG segments were found in each recording preceded by 120 s during which the heart rate varied by greater than ±2 beats/min. Specifically, the variability of heart rate was calculated as the difference between the slowest and fastest heart rate in 10-s segments of the preceding 2 min. Of these ECG segments, 100 were selected that were separated by at least 2 min from each other and of which 50 and 50 were preceded by the largest heart rate deceleration and acceleration, respectively. An additional 30 ECG segments preceded by variable heart rate were obtained from six prespecified 5-min windows during which the subjects were changing their postural positions.

Thus, in total, up to 360 ECG segments, each of 10-s duration, were selected for a detailed QT interval measurement from each long-term recording.

ECG measurements. Each selected 10-s ECG segment was filtered, and its baseline wander was removed. [Modified versions of published filtering (4) and baseline wander removal (23) techniques were used.] From the filtered signals, the median representative beats were constructed and the QT intervals (from global QRS onset to global T-wave offset) were measured using a pattern matching algorithm. In each ECG, the Q onset and T offset triggers were visually checked and, where necessary, manually corrected by two independently working cardiologists. These visual checks and measurement corrections were performed on a computer screen in median beats with superimposed images of all 12 leads in a 1-kHz resolution (cubic spline interpolation). Cardiologists were also permitted to declare an ECG as nonmeasurable.

The results of these measurement checks by the two cardiologists were compared, and the ECGs in which the cardiologists differed either in their decision on measurability or in the QT interval duration by >6 ms were identified. These ECGs were returned to the same pair of cardiologists for repeated verification. After the second measurement, the results provided by the two cardiologists were again compared and cases of disagreement reconciled by a senior cardiologist. All ECGs of the same individual were processed by the same pair of cardiologists and by the same senior cardiologist.

After obtaining these manually verified and reconciled measurements, all measured ECGs of the same subject were pattern classified with an adjustment algorithm to ensure that similar ECG morphological patterns were measured systematically. Thereafter, the measurement was again visually checked and, where necessary, manually corrected by a senior cardiologist. Subsequently, the Q onset and T offset triggers were projected from the median beats into the individual beats of the measured ECG segment (18).

For each measured 10-s ECG segment, the QRS positions were identified, and a history of ≥250 RR intervals preceding the measured segment was obtained [the number of 250 RR intervals was based on previous observations (25) and practicality]. The computer identification of the QRS complexes and of RR interval measurement was verified and, if necessary, corrected by a cardiologist on a computer screen displaying the 12-lead rhythm.

Measurement of QT/RR hysteresis. In each recording, the QT/RR hysteresis was assessed using an optimized version of a previously published methodology (25). The assessment used the data obtained from the ECG segments preceded by variable heart rates.

Presently, it is not known whether the influence of the RR interval history on the QT interval duration diminishes with every cardiac cycle or whether it decreases with time. To distinguish this, two different modes of hysteresis were investigated. The first expressed the history of the QT measurement in terms of the number of RR intervals; the other, in terms of the time elapsed. In the following text, these modes are called the "interval-based" and the "time-based" hysteresis.

As previously published, the profile of the QT/RR hysteresis was modeled by a numerical parameter of exponential decay (25), i.e., the two modes of hysteresis were characterized by numerical parameters λ_I and λ_T with the hysteresis contribution of the preceding n RR intervals to the QT interval duration weighted as follows:

$$\frac{1 - e^{-\lambda_I \frac{n}{L}}}{1 - e^{-\lambda_I}}$$

for the interval-based QT/RR hysteresis, and

$$\frac{1 - e^{-\lambda_T \frac{\sum_{i=1}^{n} RR_i}{\sum_{i=1}^{L} RR_i}}}{1 - e^{-\lambda_T}}$$

for the time-based QT/RR hysteresis, where L is the number of all RR intervals in the history $(RR_i)_{i=1}^{L}$ of the QT interval measurement.

In addition to the QT/RR hysteresis assessment in each separate recording, the data of the 10-s ECG segments preceded by the most variable heart rate were also pooled from all three repeated recordings in each subject and the same algorithms provided pooled hysteresis profile and coefficients λ_I and λ_T. Hence, for each hysteresis mode, four different values of the λ coefficients were found for each subject: three corresponding to the three separate recordings and one corresponding to the pooled data of all three recordings.

Correction for QT/RR hysteresis. Each QT interval measurement was related to four different RR-interval values. These were *1*) the averaged RR interval in the 10-s ECG segment in which the QT interval measurement was made, *2*) the average of RR intervals in 120-s history of the QT interval measurement, *3*) the RR interval value obtained from the interval-based hysteresis profile applied to the RR history of the measured segment (the profiles were calculated for each cardiac beat within the measured segment and subsequently averaged), and *4*) the same as in *3* above using the time-based hysteresis.

For each of these possibilities, the pairs of QT and RR interval data were processed with a previously described spectrum of linear and nonlinear regression models (2, 19, 25) from which the model leading to the lowest regression residual was selected.

These regression residuals (i.e., the SD of QTc values when the optimum regression model is converted into an individualized correction formula) were calculated for *1*) all the QT data measured in the same recording and *2*) all the QT data measured in the same subject (i.e., pooling the 3 separate recordings together).

Correction for heart rate. From the spectrum of linear and nonlinear regression models (2, 19, 25), the model that led to the lowest sum of regression residuals over all study subjects was identified and the slope parameter α of this model (i.e., the parameter used for heart rate correction with this population optimum model) was optimized for each subject using all the measured data. The same individual optimization of the slope parameter α was also performed for the linear model $QT = \alpha \times RR + \beta$, as well as for the parabolic log/log model $QT = \beta \times RR^{\alpha}$. These individually optimal α parameters were correlated with the individual λ_I and λ_T coefficients (obtained from pooled data of the same subject) to study the relationship between QT/RR hysteresis and the static QT/RR relationship.

Statistics and data evaluation. The RR interval measurements and the records of RR history for the purposes of hysteresis calculation were expressed in seconds. The QT intervals and QT/RR regression residuals were expressed in milliseconds.

In each subject, each of the three recordings provided separate values of λ_I and λ_T. From these three values, the intrasubject average and intrasubject SD of the λ_I and λ_T values were calculated. The intrasubject SDs were compared (1-sample, 2-tailed *t*-test) with the intersubject SD of the individual means as well as with the intersubject SD of the λ_I and λ_T values obtained from the pooled three recordings. A statistically significant difference with lower intrasubject SD was interpreted as *1*) proof of consistency in the measurement of the λ_I and λ_T values and *2*) subject-specific individuality of the QT/RR hysteresis. To test whether the comparison between intrasubject and intersubject SDs distinguishes between individual-specific values and measurement characteristics that are substantially influenced by random errors, the same comparison was also applied to the maximum noise contents found in the analyzed ECG segments of separate long-term recordings.

To test the stability of the QT/RR hysteresis assessment from long-term ECG recordings, the averages of the λ_I and λ_T values obtained from the three separate recordings in the same subject were also compared with the λ_I and λ_T values derived from the pooled QT and RR interval data of the three recordings. The correspondence between the averages and the values obtained from the pooled data was interpreted as a further confirmation of measurement stability. For the same purpose, the individual λ_I and λ_T values were mutually correlated.

To test the relationship between the QT/RR hysteresis and the static QT/RR relationship, the λ_I and λ_T values were correlated with the individual correction parameters.

The QT/RR regression residuals (i.e., the SDs of the QTc values) were compared (1-sample, 2-tailed *t*-test) for the four different possibilities of expressing the RR interval.

The λ values, their intraindividual SDs, and the QT/RR regression residuals were compared between both sexes (2-sample, 2-tailed *t*-test, assuming different variances).

Continuous data are presented as means ± SD. Statistical significance was assumed if $P < 0.05$.

RESULTS

After the exclusion of nonmeasurable segments, the study used QT interval and RR history measurements of 43,046 individual 10-s ECG segments. The averaged slowest and fastest heart rates in individual long-term recordings were 51.6 ± 6.1 and 113.7 ± 12.4 beats/min, respectively. The differences between the fastest and slowest heart rates in the individual long-term recordings were 60.0 ± 11.5 (range, 39.7–96.0) beats/min. The averaged heart rates of the analyzed ECG segments were 73.4 ± 6.7 (range per individual long term recordings, 59.0–89.1) beats/min.

Characteristics of QT/RR hysteresis. In the total population, the mean values of λ_I and of λ_T obtained from the pooled data in the same subjects were 4.68 ± 0.63 and 5.04 ± 0.70, respectively. There was a trend toward higher λ values (i.e., faster QT adaptation to heart rate changes) in males than in females (λ_I of 4.85 ± 0.59 vs. 4.48 ± 0.64; λ_T of 5.21 ± 0.66 vs. 4.82 ± 0.71), but it did not reach statistical significance ($P = 0.07$ and 0.08, respectively). Since the averaged heart rates were above 60 beats/min, λ_T values were larger than λ_I values ($P < 0.0001$), but the λ_I and λ_T values in the same subjects were highly correlated ($r = 0.899$).

Intrasubject and intersubject spread of λ values is shown in Fig. 1. The λ values were found highly individual with the intrasubject SDs (0.354 ± 0.221 for λ_I, and 0.390 ± 0.197 for λ_T) substantially smaller than the intersubject SDs of individual means of λ (0.641 for λ_I, $P < 0.0001$; and 0.711 for λ_T, $P < 0.0001$), as well as the intersubject SDs of λ values obtained from pooled recordings in the same subjects (0.631 for λ_I, $P < 0.0001$; and 0.702 for λ_T, $P < 0.0001$). High statistical significances confirming the individuality of λ values were also found when repeating these tests separately in women and in men. The intrasubject SDs of λ_I and λ_T were not different between women and men.

Figure 1 also shows the comparison of the maximum noise levels analyzed in the same way. The intrasubject SDs of maximum noise (15.6 ± 16.1 technical units) were not statistically different from the intersubject SD (19.1, $P = 0.19$).

Figure 2 shows the comparison between λ values obtained when averaging the results of the separate recordings in each subject and when pooling these recordings together. There were only negligible differences (correlation coefficient: $r = 0.988$ for λ_I and $r = 0.989$ for λ_T; differences pooled minus the average of −0.003 ± 0.098 for λ_I and 0.005 ± 0.104 for λ_T).

The population range of λ_I values was between 3.51 and 6.07, corresponding to the 50% QT adaptation to RR interval change after 48 and 28 cardiac cycles (90% adaptation after 148 and 95 cycles, respectively). Correspondingly, the population range of λ_T values was between 3.82 and 6.42, corresponding to the 50% QT adaptation to RR interval change after 44 and 27 s (90% adaptation after 140 and 90 s, respectively). The averaged λ_I and λ_T values corresponded to 50% QT/RR adaptation completed after 37 cardiac cycles and 34 s (90% adaptation after 120 cycles and 112 s).

Relationship between QT/RR hysteresis and QT/RR patterns. With both hysteresis modes, the smallest QT/RR regression residuals were on average found with the arcus hyperbolic sine regression model (2, 25) $QT = \alpha \times \text{arcus hyperbolic sine}(RR) + \beta$.

Figure 3 shows that individually optimized α parameters of this model, as well as of the linear and parabolic log/log models, were not influenced by the hysteresis mode; i.e., once the QT/RR hysteresis was considered, the same QT/RR pattern was found in each individual irrespective of whether correcting for interval-based or time-based hysteresis.

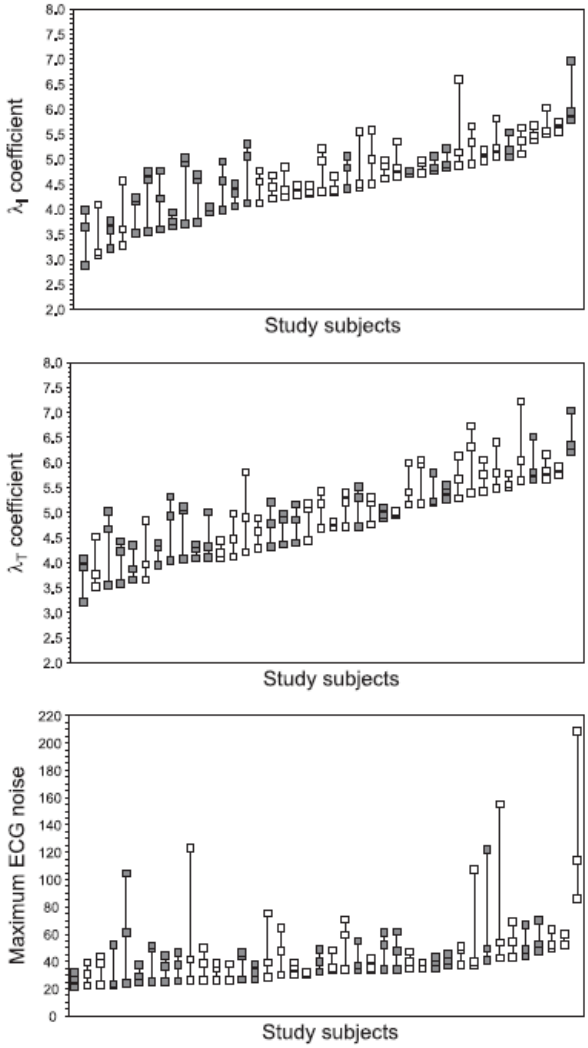

Fig. 1. Distribution of λ values measured in individual recordings of the study. *Top*, *middle*, and *bottom*: values measured in 3 repeated recordings of the same subject are shown above each other and connected by a vertical line. The study subjects were sorted according to the individually lowest values of λ and/or noise levels, and, consequently, the order of the subjects is not the same. White squares, male subjects; black squares, female subjects. *Top*: λ_I values. *Middle*: λ_T values. Note that the data reproducibility within subjects is clearly greater than it is between subjects (see main text for statistical comparisons). Note also that the distribution of the λ values shown in the *top* and *middle* is almost linear and that no plateau corresponding to more frequent "normal" values can be seen. *Bottom*: validation of the comparison test with the maximum ECG noise levels that were not consistently different between study subjects.

As previously reported (19), we found different α parameters in women and men, confirming that women have steeper QT/RR patterns. These differences between the α parameters in men and women were 0.165 ± 0.022 vs. 0.188 ± 0.014, $P = 0.0004$; 0.365 ± 0.042 vs. 0.395 ± 0.028, $P = 0.0112$; and 0.219 ± 0.026 vs. 0.246 ± 0.018, $P = 0.0004$; for the linear, parabolic log/log, and arcus hyperbolic sine regression model, respectively.

Figure 4 shows that the λ values were unrelated to the α parameters of QT/RR regression models. With all the previously published regression models (25), the correlation coefficients between α parameters and λ_I values ranged between −0.298 and 0.136, whereas between α parameters and λ_T values, the correlation range was between −0.206 and 0.207. Even without corrections for multiplicity of comparisons, none of these correlation coefficients was statistically significant.

QT/RR regression residuals. On average, the QT/RR regression residuals were slightly but statistically significantly lower when combining the optimized QT/RR regression model with individually optimized time-based hysteresis correction rather than with the individually optimized interval-based hysteresis correction.

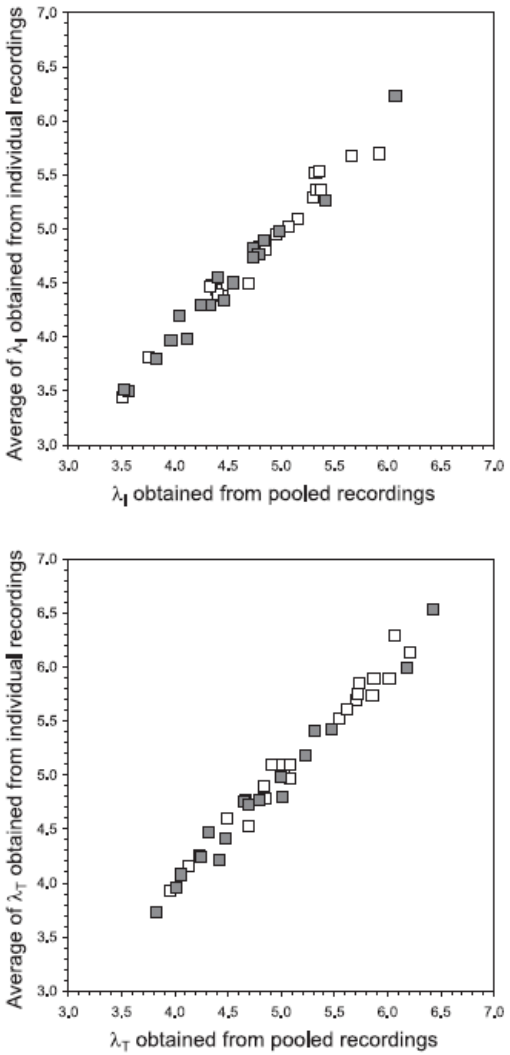

Fig. 2. Scatter diagrams of λ values obtained from the pool of the 3 recordings in the same subject (*x*-axes) plotted against the average of λ values measured in the same 3 recordings separately. *Top*: λ_I values. *Bottom*: λ_T values. Note that the two assessments lead practically to the same values (see main text for statistical tests).

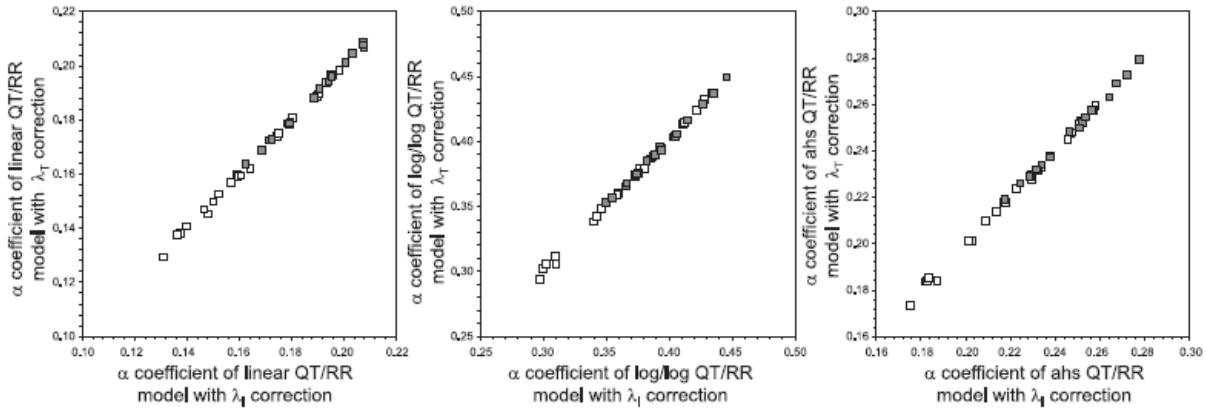

Fig. 3. Scatter diagrams of α parameters of linear (*left*), parabolic log/log (*middle*), and arcus hyperbolic sine (AHS; *right*) regression models calculated after correcting the QT/RR data for interval-based hysteresis (*x*-axes) and for time-based hysteresis (y-axes). Note that there are no differences in the correction parameters. (The same result was obtained with other QT/RR correction models that are not shown here.)

When calculating the QT/RR regression residuals in the pooled data of all three recordings in the same subject (Fig. 5), the mean residuals for 10-s RR average, 120-s average, interval-based hysteresis, and time-based hysteresis were 11.8 ± 2.16, 6.96 ± 1.41, 5.53 ± 1.13, and 5.46 ± 1.07 ms, respectively. All the differences were statistically significant ($P = 0.0023$ for the difference between the hysteresis modes, and $P < 0.0001$ for all other comparisons).

Similar differences were found when calculating the QT/RR regression residuals in each recording separately and averaging them in each subject (Fig. 6). The mean residuals for 10-s RR average, 120-s average, interval-based hysteresis, and time-based

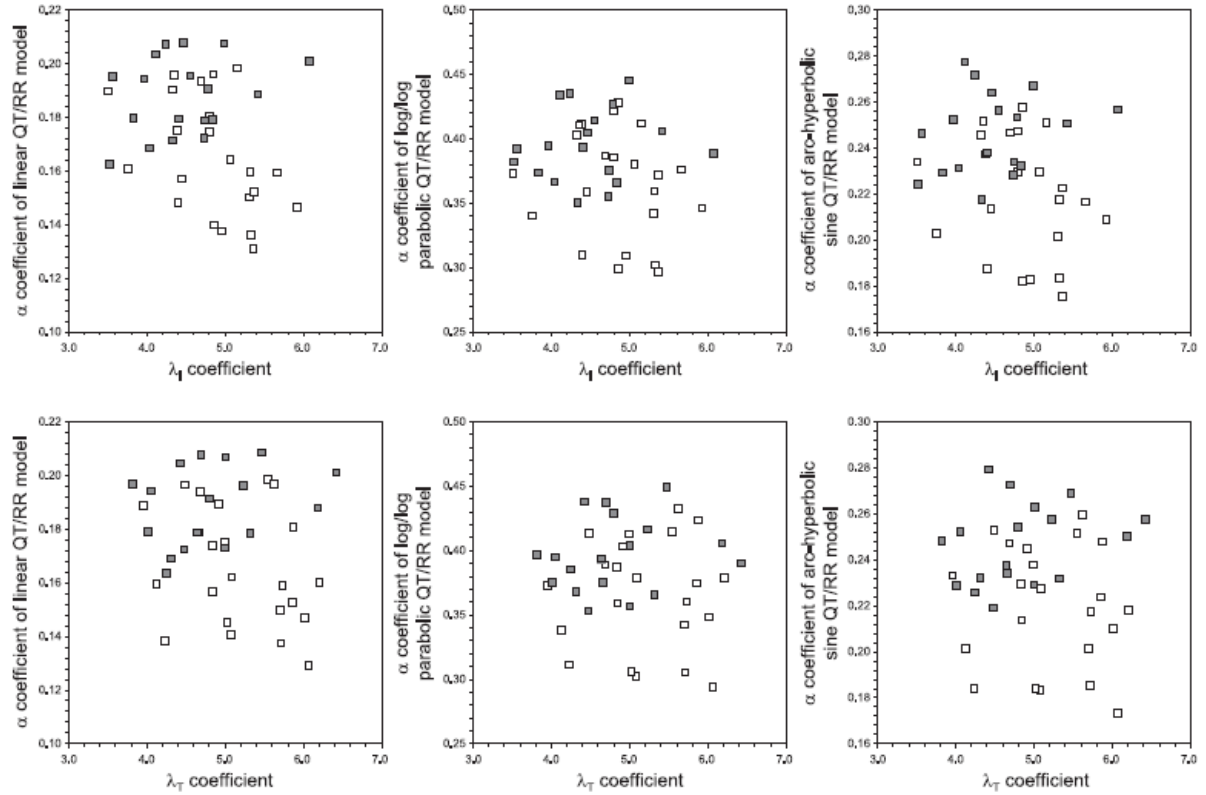

Fig. 4. Scatter diagrams of λ_I values (*top*) and λ_T values (*bottom*) plotted against α parameters of linear (*left*), parabolic log/log (*middle*), and arcus hyperbolic sine (*right*) regression models calculated after correcting the QT/RR data for interval-based hysteresis (*top*) and for time-based hysteresis (*bottom*). Note that in none of these cases are the values mutually correlated. (The same result was obtained with other correction models that are not shown here.)

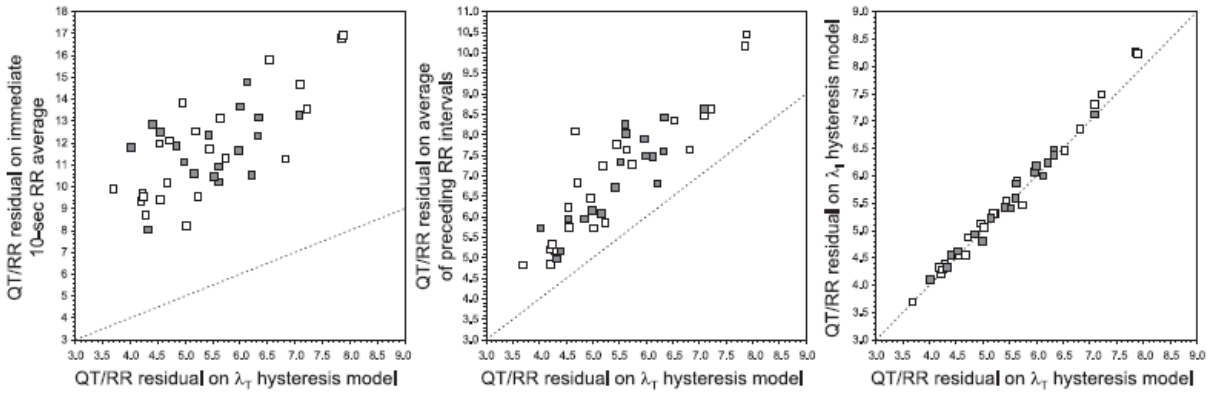

Fig. 5. Individually optimized QT/RR regression residuals [i.e., SDs of individually calculated corrected QT (QTc) values] calculated after correcting the QT/RR data for time-based hysteresis plotted against the QT/RR residuals with RR values taken from 10-s RR averages (*left*), QT/RR residuals with RR values taken from the 120-s RR interval averages (*middle*), and QT/RR residuals with RR values taken from the correction for interval-based hysteresis (*right*). All the regression residuals were calculated in pools of the 3 recordings made in the same subject. Dashed lines show the identity.

hysteresis were 11.36 ± 2.00, 6.33 ± 1.31, 4.75 ± 0.93, and 4.66 ± 0.85 ms, respectively. All the differences were again statistically significant ($P = 0.0016$ for the difference between the hysteresis modes, and $P < 0.0001$ for all other comparisons).

DISCUSSION

Three principal conclusions can be made from this study. First, the QT/RR hysteresis can be reliably and consistently assessed from the long-term ECG recordings.

Second, similar to the static QT/RR relationship, the dynamic patterns of QT/RR hysteresis show substantial intrasubject stability with a high intersubject variability. Although we have not obtained identical λ values in repeated recordings of the same individual, the intrasubject spread was remarkably smaller than the spread in the population. Not only were the differences highly statistically significant, but intersubject SDs were practically twice as large as intrasubject SDs.

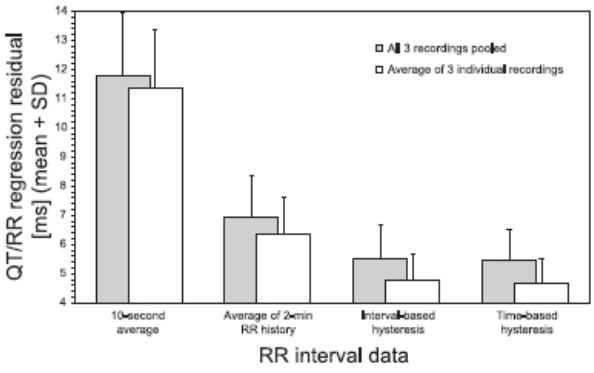

Fig. 6. Graphical representation of the statistical comparisons (see main text for exact values and statistical tests) of individually optimized QT/RR residuals (i.e., SDs of individually calculated QTc values). The individual bars correspond to QT/RR residuals with RR values taken from 10-s RR averages, RR values taken from the RR interval averages of the preceding rhythm, RR values taken from the correction for interval-based hysteresis, and RR values taken from the correction for time-based hysteresis. Gray bars, pools of the 3 recordings made in the same subject; white bars, averaged values of the QT/RR residuals obtained in each of the same recordings separately. Population mean values and SDs are shown.

Third, it appears that the static QT/RR relationship, which is by how much the QT interval shortens or prolongs when cardiac cycles reach stable faster or slower rate, and the QT/RR hysteresis, which is how quickly the QT interval adapts to heart rate acceleration or deceleration, are two different and unrelated physiological processes. Both these processes are individually specific to separate subjects. Their population assessment suggests that there are no combinations that would be more normal or physiological than others (see the spread of values in Fig. 4).

In addition to these conclusions, we also observed statistical differences between the two hysteresis modes. When using the QT/RR residual as an arbitration, the time-based QT/RR hysteresis led to lower QTc variability than interval-based hysteresis. It thus seems that the influence of the heart rate history on the QT interval duration diminishes more likely with the time elapsed rather than with the number of cardiac cycles.

As far as we are aware, this is the first study investigating the individuality of QT/RR hysteresis in healthy subjects. Consequently, there is little published data with which we can compare our principal results. Nevertheless, the averaged λ values agree well with the previously reported adaptation of both monophasic action potentials and surface QT intervals in response to abrupt changes in pacing rate (11, 17). With both these experiments, 90% adaptation was reported after ~2 min (11, 17), whereas the mean value of λ_T found in our study corresponds to 90% of adaptation after 112 s.

The individuality of the hysteresis profiles also agrees well with the observed individuality of other dynamic ECG parameters that were reported not only for QT/RR profiles (2, 19) but also for heart rate dependencies of other ECG intervals (22). Indeed, in consideration of the intrasubject stability of the hysteresis profiles, the spread of values in Fig. 4 resembles fingerprint-like differences. The identification of the mechanisms responsible for such stable intersubject differences is well beyond the scope of this investigation. We can only speculate that the observed differences between subjects are a manifestation of highly individual distributions of ionic chan-

nels and of their interactions in different cardiac tissues. It is likely that the individual ECG phenotype is related to the intersubject genotype differences. However, it is equally plausible that the details of ionic channel distributions and interactions are caused by congenital embryonic chance rather than genetically determined. Future studies are needed to address these possibilities, e.g., by comparing high-precision electrocardiography in monozygotic and dizygotic twins.

The values of the QT/RR regression residuals (i.e., SDs of the individually heart rate- and hysteresis QTc intervals) also confirmed the minimum spread and little variability of QTc values in healthy subjects that our group has recently reported in an independent population (20). Although there is a circadian pattern of QTc intervals during the daytime hours (e.g., due to postprandial changes), the variability of QTc values when accurately measured and corrected is much smaller than previously reported (13, 24, 27).

The study has several important practical implications. Scrambling the effects of the dynamic QT/RR hysteresis with the effects of the stable QT/RR relationship (10, 16, 30) into one descriptor of repolarization dynamics seems counterproductive. Rather, both physiological processes should be measured and characterized separately and only subsequently combined in studies of QTc interval dynamics. The very substantial reduction of SD of QTc values achieved with the combination of individual hysteresis and individual heart rate correction suggests that both these corrections should be used in accurate studies investigating QTc changes. This concerns not only physiological influences of the QTc interval by various provocations but also the studies of drug-induced QTc changes (6). The power of such investigations will be substantially increased (and thus the necessary study size decreased) if the combined individual-specific corrections for both heart rate and hysteresis are used. Correcting QT interval individually for both rate and hysteresis appears clearly preferable to preselecting ECG data in which the effect of QT/RR hysteresis is considered unimportant because of heart rate stability (8).

Further detailed facets should be researched to characterize the physiology of QT/RR hysteresis more fully. Among others, we have not investigated whether the hysteresis profiles are different when QT interval durations respond to heart rate accelerations and decelerations. Similarly, the influence of heart rate levels is not known, i.e., whether the hysteresis profile is the same for, say, heart rate acceleration between 50 and 60 and between 80 and 90 beats/min.

A number of limitations of this investigation should also be considered. Our data were restricted to daytime hours in only 40 subjects. It is well known that the QT/RR relationship is different during the day and night (7, 15), and the same might apply to QT/RR hysteresis. The restriction of our data prevents us from commenting on such a possibility. Furthermore, we have confirmed the individuality of the QT/RR hysteresis by studying parameters of the exponential decay model. Although this model was previously reported in a study of cardiac patients (25) and corresponds well to the patterns previously observed in experimental studies with fixed rate pacing (11), it is not guaranteed that it is the truly optimum model for the description of QT/RR hysteresis in healthy subjects. It is possible that with different models, the intrasubject stability would be further increased. In other words, the individuality of the hysteresis can only be stronger compared with what we found in this investigation. Despite the validation of the statistical evaluation of intra- and intersubjects SDs with the maximum noise levels, standard statistical analysis of variance would have been preferable. The data restriction to three repeated recordings in each subject prevented us from employing these standard tests. A different model might also influence our conclusion on the difference between interval- and time-based hysteresis. Also, the range of ages of the subjects of this study was rather narrow, and, therefore, we were unable to relate the hysteresis profiles to age. Similar to other regulatory mechanisms (5, 9), the influence of age might be expected. In addition, the population of the study was not massive, which to some extent restricted the statistical power. It is possible that with a larger number of subjects, statistically significant sex differences in the hysteresis profiles would be found. In any case, however, these would not be as marked as the differences in QT/RR profiles (19) and in the heart rate correction parameters that we easily detected in this investigation. Since we have neither distinguished QT/RR hysteresis linked to heart rate acceleration and deceleration nor studied hysteresis changes during the day, we are unable to comment on the possibility of such differences. Finally, several of the numerical parameters used during ECG processing (e.g., the 6-ms agreement limit for separate readings between the two cardiologists) were derived from previous experience (21), as well as reflecting an ECG measurement practicality. We are unable to comment on the influence of these particular settings on the overall ECG measurement process.

Despite these limitations, the investigation shows very convincingly that the profile of QT/RR hysteresis, i.e., the speed with which the QT interval adapts to heart rate changes, can be reliably assessed in long-term ECG recordings. The profile of the hysteresis is highly individual with intrasubject stability and intersubject variability. The characteristic of QT/RR hysteresis is independent of the static QT/RR relationship and should thus be treated as a separate physiological process. The combination of the individual-specific heart-rate correction with the individual-specific hysteresis correction of the QT interval is likely to lead to substantial improvements in the precision of studies of cardiac repolarization.

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Práce se věnuje popisu méně obvyklých deskriptorů repolarizace: prostorové smyčce vektoru vlny T a prostorovému úhlu tohoto vektoru k vektoru komplexu QRS (QRS-T angle) ve srovnání s chováním intervalu QT. Výsledky ukazují, že parametry vlny T reagují na změny tepové frekvence rychleji ve srovnání s intervalem QT a tedy se může jednat o fyziologicky rozdílné procesy.

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Dynamic properties of selected repolarization descriptors

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Abstract A number of morphological indices have been proposed to characterize electrocardiographic patterns of ventricular repolarization mostly studying spatial and temporal patterns of T waves. Comparisons between different clinical populations exist but data on the suitability of the T-wave descriptors to characterize and quantify physiologic regulations of ventricular repolarization are lacking. To initiate such investigations, a study was conducted comparing the influence of provoked heart rate changes on the duration of QT interval, the roundness of T wave loop expressed by the relative T wave area, and on the 3-dimensional QRS-T angle. A population of 40 healthy subjects (18 women, mean age 30.4 ± 8.1 years) was studied. In each subject, provocative tests involving changes from strict supine to unsupported sitting and to unsupported standing positions were repeated twice during each of 3 separate monitoring days. Continuous 12-lead electrocardiograms were obtained during the provocative tests. The speed of the adaptation of the repolarization descriptors to heart rate changes was characterized by λ parameters of previously published exponential decay model of R-R interval related hysteresis. The comparisons showed that the adaptation of QT interval to heart rate changes was much slower than that of the investigated T-wave morphological descriptors: the mean (SD) values of λ parameters were 5.01 ± 1.13, 12.72 ± 8.66, and 12.90 ± 11.37 for QT interval, QRS-T angle, and relative T-wave area, respectively ($P < .001$ for the difference between QT interval and both morphological descriptors). The study suggests that the different numerical quantifiers of vertricular repolarization that may be derived from standard electrocardiographic tracings likely represent separate and distinct physiologic entities.

Introduction

Detailed electrocardiographic characterization of ventricular repolarization is still a matter of debate. In addition to the simple measurement of electrocardiographic (ECG) intervals, such as the QT interval, the T peak–to–T end interval, JT interval, and others,[1-3] a number of numerical characteristics have been proposed with the aim of gaining further insight into the physiologic properties of repolarization processes as well as into the distinction between normal and abnormal repolarization in different clinically well defined populations. These additional measures range from simple calculations of areas under the T wave in selected electrocardiographic (ECG) leads[4] up to the 3-dimensional (3D) reconstruction of the vectorcardiographic T-wave loop (mostly using the algorithms of singular value decomposition) and quantification of its character.[5,6]

Several of these novel characteristics of ventricular repolarization were reported to carry diagnostic and prognostic information not only in patients with congenital repolarization abnormalities[7-9] but also in patients with acquired cardiac disease such as survivors of acute myocardial infarction[10] or more generally in patients with documented ischemic heart disease.[11] Some of the measures were also investigated in various epidemiologic studies and reported to carry prognostic information in general population.[12-14]

In spite of this progress in applied cardiography, the detailed understanding of repolarization electrophysiology is much less advanced. The relationship between the different ECG-based characteristics is largely unknown and it has not even been firmly established whether the different descriptors relate to different facets of the same physiologic processes or whether they represent different attributes with distinct physiologic background.

To fill this gap in our knowledge, focused physiologic studies are needed concentrating on the behavior of different repolarization characteristics under various conditions.

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Hoping to contribute to this field, we are reporting on a study that investigated selected descriptors of ventricular repolarization in a population of healthy subjects of both sexes and studied their adaptation to heart rate changes due to autonomic provocations.

Methods

Population

For the purposes of the investigation, data were available from a sample of 40 healthy subjects (18 females, mean age 30.4 ± 8.1 years). Male subjects were marginally younger (28.9 ± 6.7 years) than female subjects (32.3 ± 19.4 years) but this difference did not reach statistical significance. At the study onset, all subjects had a normal physical examination and normal resting 12-lead ECG. The investigation was a part of a larger study that was approved by the ethics committee of the clinical facility. All subjects gave informed written consent.

Investigation

Each subject was studied at 3 separate days with gaps of approximately 3 weeks between repeated sessions. Constant environmental conditions of the investigations were strictly maintained.

At each session, each subject underwent two provocative tests repeated after approximately 2 hours. The first and second tests were always performed at the same time of the day in the given investigation session.

One half of the population performed provocative postural test composed of 10 minute strict supine position followed by 10 minutes of undisturbed and unsupported sitting position, followed by 10 minutes of strict motionless standing position, followed by 10 minutes of strict supine position (test 1). The other half of the population performed similar provocative tests using a different order of the positions, namely, 10-minute strict motionless supine position followed by 10 minutes of motionless standing, followed by 10 minutes of unsupported disturbed sitting, followed by 10 minutes of motionless supine position (test 2). In each test, changes between the individual positions were completed within approximately 10 seconds. During the tests, the subjects were kept free of any external disturbance and visual contact between study subjects investigated at the same time in the same room was avoided. The subjects were not permitted to fall asleep while in the supine positions.

Electrocardiographic recordings

During the tests, continuous 12 lead electrocardiograms were obtained using SEER MC Version 2 recorders (GE Healthcare, Milwaukee, WI) with signals stored electronically for further processing. Mason-Likar positions of ECG leads were used and exactly the same lead placements were reproduced in each subject during the repeated sessions.

Electrocardiographic processing

The ECGs that were initially recorded with a 250 Hz sampling frequency were spline interpolated and resampled at 1 KHz. The continuous recordings were divided into 10-second segments with each subsequent segment overlapping the previous segments by 5 seconds. In each such 10-second segment, individual QRS positions were identified and representative median waveforms constructed for each ECG lead.

In each segment, the average R-R interval was calculated. In the representative median waveforms, the global QT interval was measured using the pattern matching algorithm. These measurements were subsequently visually checked and manually corrected using detailed quality assurance and reconciliation system as previously described.[15,16]

The representative median waveforms of each 10-second segment were further processed by singular value decomposition to obtain optimized 3D representation of the QRS and T-wave loops.[17] From these, the 3D angle between the QRS and T-wave loops was calculated and expressed in degrees, and from the 2-dimensional projection of the T wave loop, the roundness of the loop was expressed using the concept of the relative T wave area. Calculation was based on previously published technology.[17,18]

Data evaluation

The ECG recordings obtained in repeated tests of the same subject were synchronized in respect of the beginning of the test and average values of R-R interval (or heart rate), QT interval, QRS-T angle, and the relative T-wave area were obtained for each 10-second interval of the 40-minute period of the test, moving these intervals in 5-second steps. Subsequently, these sequences were aligned for different subjects and in the subgroups of women and men who performed provocative test 1 and 2, the absolute values of the measured indices were averaged and displayed including the standard errors of mean within the given groups of subjects.

In each subject, the difference in the measured parameters was also calculated from the average of the baseline value within the initial 10-minute supine period, and these relative changes were again obtained for each 10-second interval in 5-second steps. These relative changes of the investigated parameters were displayed in the same way as the absolute values.

To investigate the dynamic changes of QT interval, QRS-T angle, and relative T-wave area in response to the provoked heart rate changes, hysteresis loops were constructed between the group averages of R-R intervals and group averages of the repolarization related parameters. These constructions were made for the groups of women and men performing tests 1 and 2 and used sequential scatter diagrams between the time synchronized sequences of mean R-R intervals and mean values of repolarization parameters. The widths of the hysteresis loops were judged visually.

To express the hysteresis profile of the individual T wave parameters numerically, previously published exponential decay model[16,19] was used in combination with linear

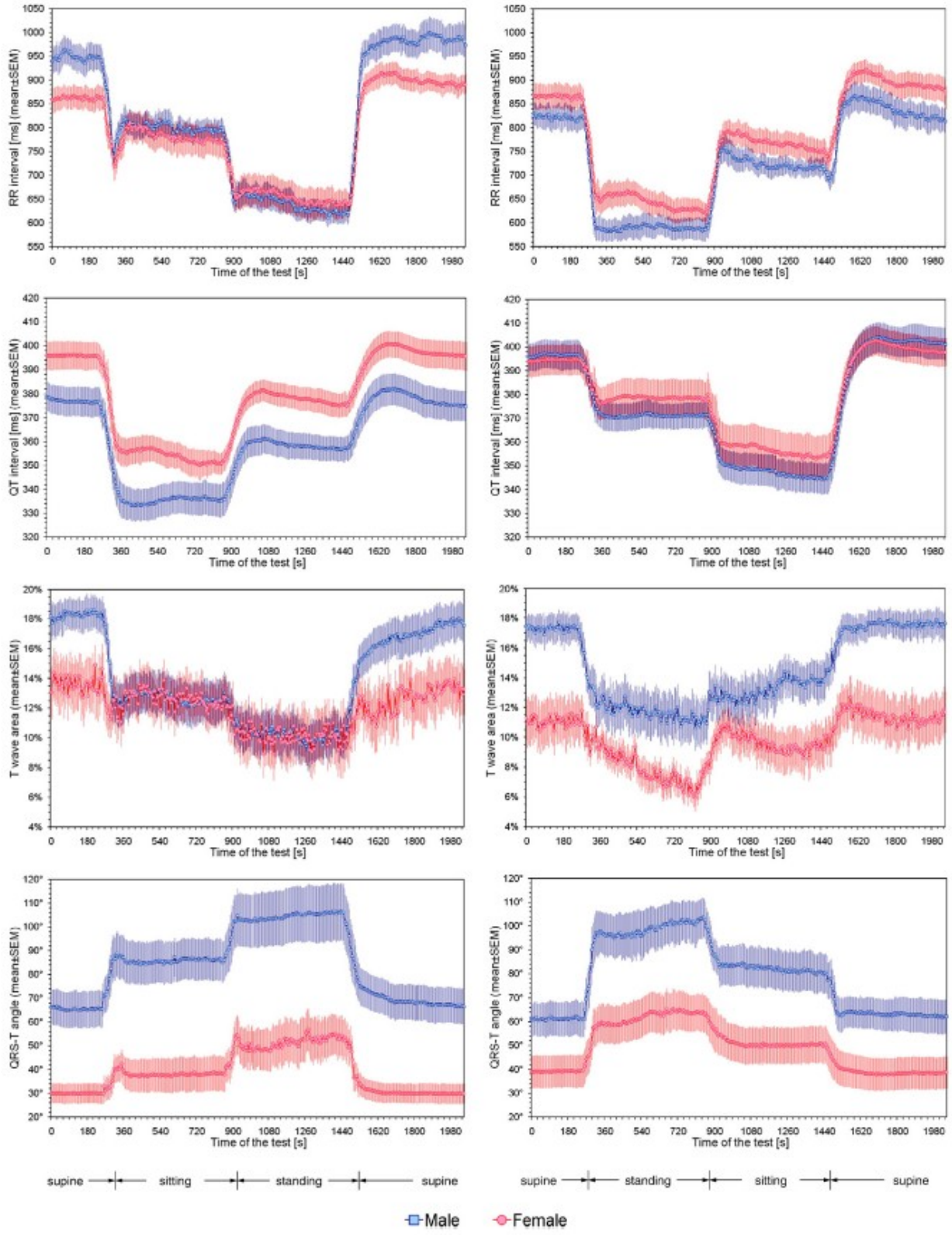

Fig. 1. Changes in absolute value of measured parameters during the provocative tests. In each panel, mean values in subgroups of women and men are shown together with the standard error of mean. The values are shown for individual 10-second segments moved in 5-second steps. The timing of the displays starts in the middle of the initial supine position of both provocative tests. The left and right panels correspond to provocative tests 1 and 2, respectively. Red (lighter) and blue (darker) marks correspond to women and men, respectively.

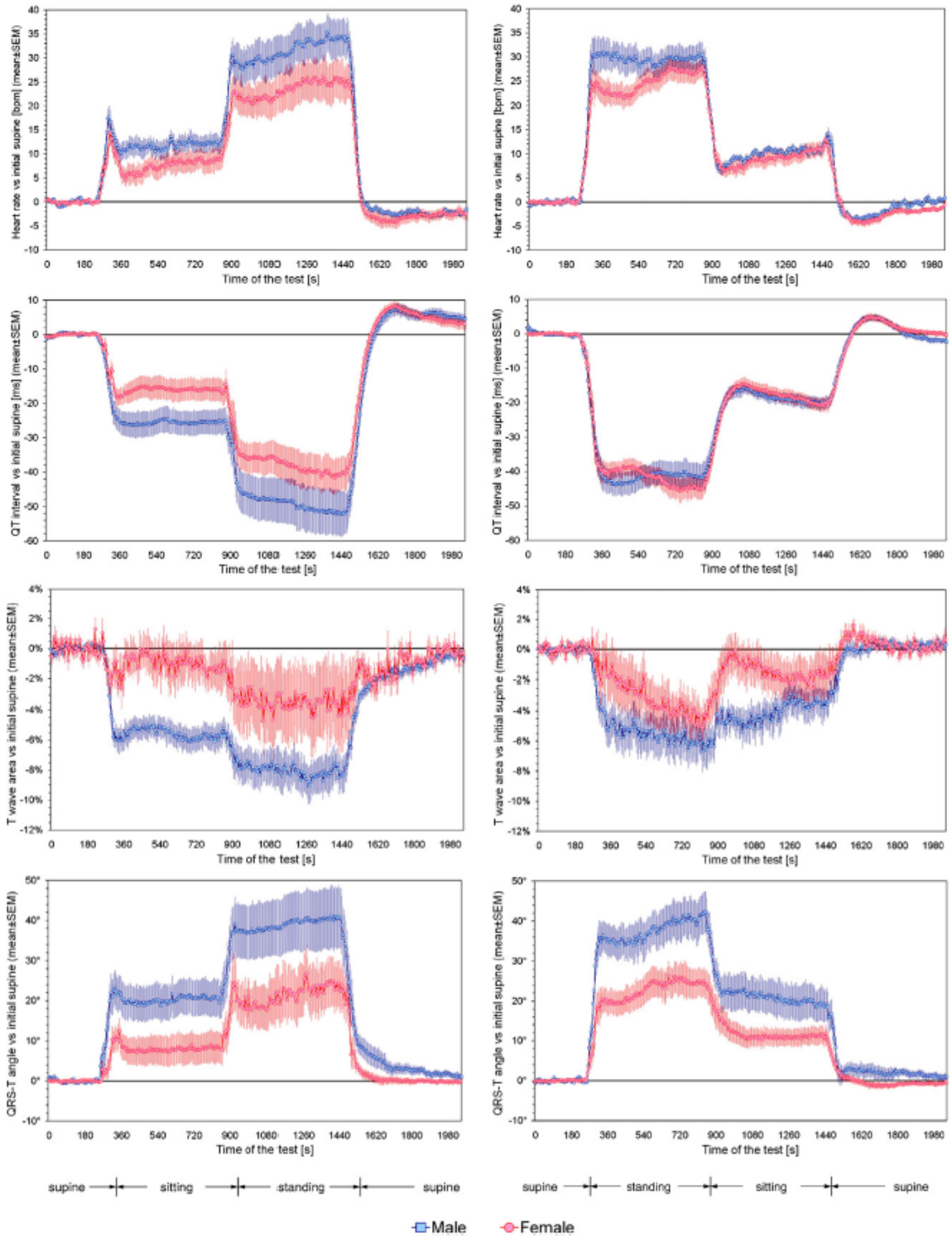

Fig. 2. Relative changes in measured parameters during the provocative tests. In each subject, the relative change was calculated as the difference from the individual average of the values measured during the initial supine position of the test. In each panel, mean values in subgroups of women and men are shown together with the standard error of mean. The values are shown for individual 10-second segments moved in 5-second steps. The top panels show heart rate changes, the heart rate values were derived from the measured values of 10-second averages of R-R intervals. The timing of the displays starts in the middle of the initial supine position of both provocative tests. The left and right panels correspond to provocative tests 1 and 2, respectively. Red (lighter) and blue (darker) marks correspond to women and men, respectively.

regression model between each T wave parameter and R-R interval. For each subject and for each of the three T-wave parameters, a hysteresis coefficient λ was found leading to the lowest regression residual of the linear regression model.

Scatter diagrams of the hysteresis coefficients λ for the different repolarization parameters were constructed using the individual values in separate study subjects.

Statistics

The values of hysteresis coefficient λ were compared between different study subgroups and between different repolarization parameters using unpaired and paired 2-tailed *t* tests. Pearson correlation coefficients were used to evaluate the relationship between hysteresis coefficients of different repolarization descriptors. $P < .05$ was considered statistically significant.

Results

Provocative tests 1, that is, the postural sequence supine → sitting → standing → supine was followed by 8 women and 12 men, whereas the provocative test 2, that is, the sequence supine → standing → sitting → supine was followed by 10 women and 10 men.

Absolute and relative values of the investigated parameters are shown in Figs. 1 and 2. Somewhat surprisingly, the postural changes between supine and standing positions

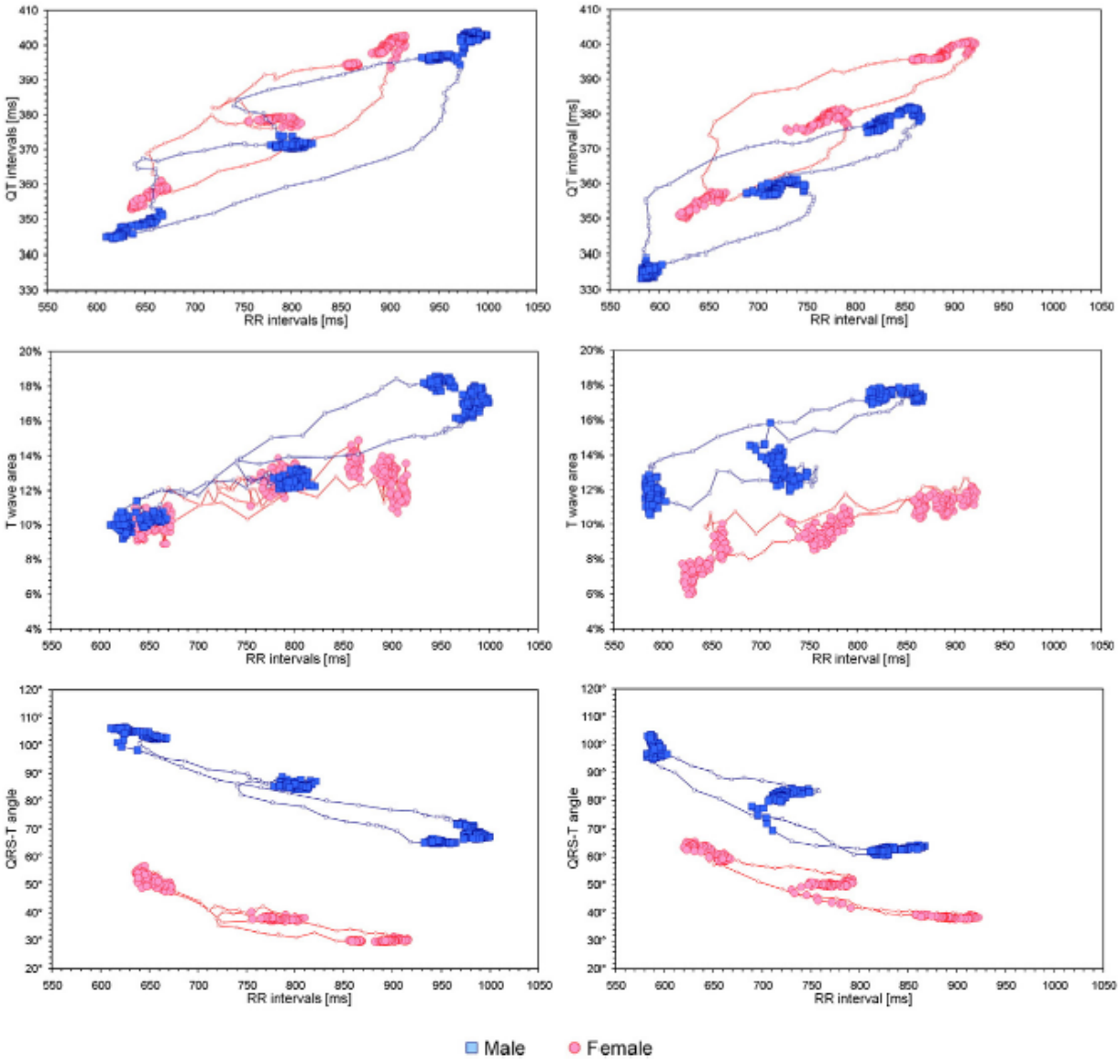

Fig. 3. Hysteresis loops of the R-R interval related changes of repolarization parameters. Each panel shows sequential scatter diagrams between mutually time-wise corresponding 10-second averages of R-R intervals and the repolarization descriptors measured in median representative beats of 10-second ECG segments. The large closed marks correspond to the relatively stable episodes of individual postural positions, the small open marks correspond to the transition periods. Red (lighter) and blue (darker) marks correspond to women and men, respectively.

led to fairly substantial differences in heart rate (Fig. 2). The graphs in Figs. 1 and 2 also indicate that the QT interval duration followed the heart rate changes with a more prolonged time lag than the other repolarization parameters.

This observation was confirmed by the construction of the hysteresis loops shown in Fig. 3. While the hysteresis loops of the QT interval were relatively wide, the hysteresis loops of the relative T wave area and, in particular, of the QRS-T angle were much narrower indicating much faster responsiveness of these repolarization descriptors to the provoked heart rate changes.

The graphs in Figs. 1–3 also show that the calculations of the relative T wave area was less stable than the measurements of the QT interval and calculation of the QRS-T angle which produced much smoother graphs.

The difference between the heart rate hysteresis of the QT interval and of the other repolarization descriptors was documented objectively by statistical comparison of the numerical hysteresis coefficients. Although we have not observed any differences in these coefficients between the populations performing tests 1 and 2 and also no statistical significant differences between women and men, the values of the λ parameter of the heart rate hysteresis of the QT interval, QRS-T angle, and relative T wave area were 5.01 ± 1.13, 12.72 ± 8.66, and 12.90 ± 11.37, respectively (complete calculations were not available in 7 subjects in whom the exponential decay model of the relative T-wave area did not converge). The differences between the λ parameters of the QRS-T angle hysteresis and of the relative T wave area hysteresis were not statistically significant but the λ coefficients of the QT/heart rate hysteresis were statistically significantly lower than those of the other factors ($P < .001$ in both cases).

Fig. 4 shows the scatter diagrams between the individual λ coefficients corresponding to the different repolarization descriptors. Substantial spread of the values in the population is obvious and the figures also show that hysteresis factors were unrelated between the different repolarization parameters. This was confirmed by the correlation of Pearson correlation coefficient (QT interval λ vs relative T wave area λ, $r = 0.115$; QT interval λ vs QRS-T angle λ, r = 0.332, QRS-T angle λ vs relative T wave area λ, $r = 0.264$; all $n = 33$). None of these was statistically significant.

Discussion

The result of the study suggests that during autonomic provocations, the investigated repolarization characteristics exhibit remarkably different dynamics. Although the QT interval adapts to the changes in heart rate rather slowly, the adaptation of the other investigated factors, and of the QRS-T angle in particular, appeared to be much faster with only very little time delay. This might suggest that the 3-dimensional properties of the T-wave loop are related to a principally different physiologic processes than those driving the duration of ventricular action potentials and translating into the duration of QT interval. The nature of such underlying physiological processes is well beyond the scope of our present study and can only be, at present, speculated on.

As far as we are aware, there are no other studies available with which we could compare these principal results of this investigation. Nevertheless, secondary observations, such as the sex difference in the 3D QRS-T angle and its heart rate dependency, are in good agreement with previous publications and data obtained from very different populations.[20,21]

Some of our observations also suggest hypotheses that might be worth investigating in more focused way in subsequent studies. For instance, a detailed comparison of the hysteresis loops shown in Fig. 3 appears to suggest that there are hysteresis differences between heart rate acceleration and deceleration and that these differences are more pronounced for the rapid adaptation of the QRS-T angle than for the slower adaptation of the QT interval duration.

This observation might actually help with the interpretation of our results. When describing the heart rate driven hysteresis of the different repolarization factors, we are not

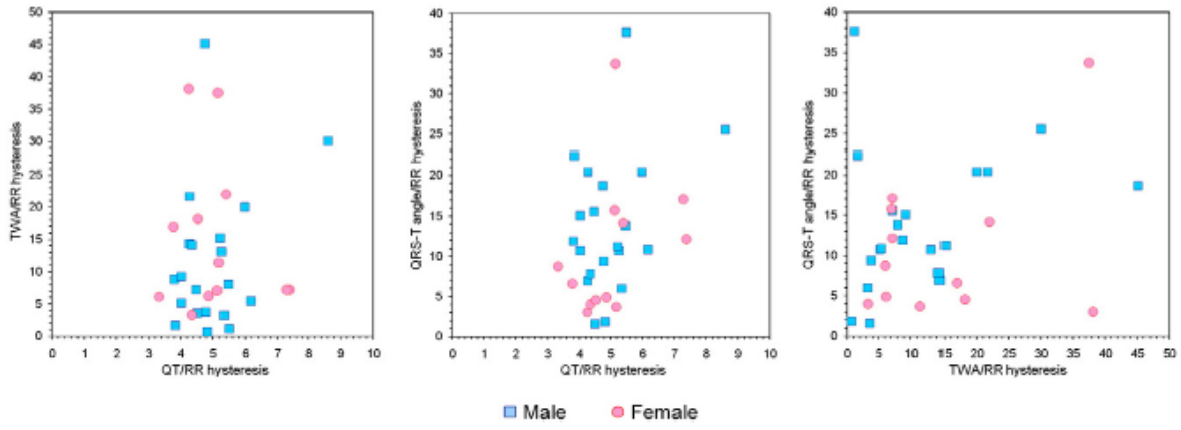

Fig. 4. Scatter diagrams of individually optimized values of the parameters λ of R-R interval drive hysteresis profiles of individual repolarization parameters. Red (lighter) and blue (darker) marks correspond to women and men, respectively. TWA, relative T wave area.

necessarily proposing that the heart rate is the only covariate which drives the properties of ventricular repolarization. Postural provocative maneuvers lead to marked changes in sympathovagal balance which is the true background of the heart rate change. It is possible if not likely that the autonomic changes have direct influence on ventricular repolarization independent of their influence on heart rate and that vagal and sympathetic surcharges influence ventricular repolarization not only in an opposite way but also with a different dynamics.

Although this study seems to provide a useful guide for further investigations, it also suffered from important limitations that need to be considered. Different samples of subjects were investigated in postural test 1 and 2. Since there has been previously described that the characteristics of repolarization dynamics are highly individual, we cannot be absolutely certain that the inter-subject differences did not influence our results. Future studies of this kind should avoid using parallel comparisons between provocative tests more active on sympathetic and vagal charges. Cross-over design should provide better comparisons. Individual-specific relationship of different repolarization descriptors to heart rate has been little studied.[22,23] We have therefore used only simple linear models between R-R intervals and the QRS-T angle and relative T-wave area. These linear regressions are not necessarily optimal in all cases and might have contributed to some increased variability in our data. It is also not obvious whether the exponential decay model that was previously found to characterize well the QT/R-R hysteresis[16,19] is appropriate for the study of the hysteresis of the other repolarization characteristics. It is very unlikely that the fairly large difference between the hysteresis characteristics found for the QT interval and for the other repolarization factors was caused by this model (since the difference is very obvious without any mathematical elaborations directly from the hysteresis loops shown in Fig. 3), but the design of a more accurate technology for studying the heart rate hysteresis of various ECG based factors is clearly warranted. Indeed, although Fig. 3 may suggests differences in the speed of heart rate adaptation between the QRS-T angle and the relative T-wave area, we have not documented this numerically, possibly because of poorly focused technology. Finally, although we can only speculate on the differences between the dynamics due to heart rate acceleration and heart rate deceleration, the data of this study and the analytical techniques at our disposal were not sufficiently robust to determine these differences objectively. In addition, we have selected the QRS-T angle and relative T-wave area since these were previously proposed to carry prognostic risks in cardiac patients.[10,18] The dynamics of other repolarization parameters (among the large spectrum of previously published suggestions) might also deserve similar investigations.

In spite of all these limitations, the study seems to document convincingly that the dynamics of different ECG based descriptors of ventricular repolarization is very different. This suggests that the different numerical quantifiers that can be derived from standard ECG tracings likely represent separate and distinct physiologic entities.

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2.1.3. Klinická diagnostika syndromu dlouhého intervalu QT – komentář k současným poznatkům

Jak již bylo uvedeno výše, diagnóza LQTS je klinická, a to přes veškerý pokrok v molekulárně genetické diagnostice. Vzhledem k tomu, že klidové hodnoty intervalu QT mohou být normální, byla navržena řada provokačních testů. Na našem pracovišti dlouhodobě využíváme bicyklové ergometrie, pilotní data jsme publikovali v roce 2000 (18). Tento přístup byl potvrzen dvěma nedávno publikovanými studiemi. Ty potvrdily, že hodnota QTc nad 480 ms ve 4. minutě restituce ergometrického testu má 100% senzitivitu pro identifikaci nosičů mutací genů *KCNQ1* a *KCNH2*, které jsou u pacientů s diagnózou LQTS nalézány nejčastěji (19, 20). Výsledky obou studií vedly dokonce k úpravě Schwartzova diagnostického skóre (Tab 1) (21).

Tab 2. Skóre pro diagnostiku LQTS (upraveno podle Schwartz et al., Circulation 2011)

		Body
EKG nálezy		
A. QTc (dle Bazetta)	≥480 ms	**3**
	460-470 ms	**2**
	450 ms u mužů	**1**
B. QTc ve 4. minutě restituce zátěžového testu ≥480 ms		**1**
C. Torsades de pointes		**2**
D. Alternans T vlny		**1**
E. Dvouvrcholová T vlna ve 3 svodech		**1**
F. Nízká TF pro danou věkovou skupinu (u dětí)		**0,5**
Osobní anamnéza		
A. Synkopa	při námaze	**2**
	bez vazby na námahu	**1**
B. Vrozená hluchota		**0,5**
Rodinná anamnéza		
A. Jasná diagnóza LQTS u přímého příbuzného		**1**
B. Náhlé úmrtí přímého příbuzného před 30. rokem		**0,5**
Hodnocení: **≤1 bod** – nízká praděpodobnost diagnózy **1,5-3 body** – střední praděpodobnost diagnózy **≥3,5 body** – vysoká praděpodobnost diagnózy		

Otevřenou otázkou zůstává popis zátěžově závislého chování T vlny. Publikovali jsme pozorování zátěžově závislého rozvoje dvouvrcholové morfologie T vln (viz přiloženou publikaci níže). Souhrnně pak byla publikována data celého našeho souboru (viz další přiloženou publikaci).

Novotný T, Šišáková M, Kadlecová J, Floriánová A, Semrád B, Gaillyová R, Vlašínová J Chroust K, Toman O. Occurence of notched T wave in healthy family members with the long QT interval syndrome. Am J Cardiol 2004;94:808-811.

IF 3,14. Citováno 2x ve Web of Science.

(publikovaná původní práce - kvantitativní podíl uchazeče 30 % - koncept, koordinace práce zúčastněných pracovišť, vyšetřování pacientů, analýza dat, text publikace)

Práce popisuje zátěží vyvolaný vývoj dvouvrcholových vln T, jejich různé morfologie a korelaci s geneticky potvrzenou diagnózou LQTS. Výsledky naznačují, že některý typ dvouvrcholových vln T může být oproti původním předpokladům obecným diagnostickým znakem více genetických typů LQTS (tedy nejen LQTS2).

Occurrence of Notched T Wave in Healthy Family Members With the Long QT Interval Syndrome

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The notched T wave is considered 1 of the diagnostic signs of long QT interval syndrome (LQTIS). The investigators report observations of notched T waves in noncarrier members of families with LQTIS and compare the exercise-induced dynamic behavior of these complex T-wave patterns in mutation carriers and noncarriers of 3 families with LQTIS. ©2004 by Excerpta Medica, Inc.

(Am J Cardiol 2004;94:808–811)

The aims of this study are to determine the prevalence and specificity of notched T waves in LQT1 and LQT2, to assess rate-dependent (exercise-induced) characteristics of notched T waves, and to correlate types of notched T waves to LQT1 and LQT2 genotypes.[1] Here, we report our preliminary observations of the occurrence of T- and U-wave fusion mimicking notched T waves in healthy members of families with long QT interval syndrome (LQTIS).

• • •

The study population included 18 members of 3 unrelated families (family 1: 6 female and 3 male members; family 2: 3 female and 2 male members; family 3: 2 female and 2 male members) with genetically established diagnosis of LQTIS (Table 1). In family 1, 5 patients were carriers of KCNQ1 mutation G973A; in family 2, 1 patient was a carrier of KCNQ1 mutation C926T;[2] and in family 3, 3 patients were carriers of HERG-KCNH2 mutation C1600T. In each family, ≥1 patient presented with syncope or aborted cardiac arrest. At the time of investigation, only 1 patient was on a β blocker. The control group consisted of 18 age- and gender-matched healthy patients. Informed consent was obtained from all the investigated patients.

All patients underwent bicycle ergometry to obtain electrocardiographic recordings in different adrenergic states. The Mason-Likar modification of standard 12-lead electrocardiography was used, and the electrocardiograms were stored electronically (CardioSys version 2.5, Marquette Hellige GmbH, Freiburg, Germany). The protocol started from a workload of 0.5 W/kg and was increased by 0.5 W/kg every 3 minutes to reach the heart rate estimated for submaximal workload according to age and gender. A macroscopic T-wave pattern was assessed in all 12 leads by 2 blinded, independent observers before exercise, at peak exercise, and in the first and sixth minutes of rest. The definition of T-wave morphology was adapted from Lehmann et al[3] and Lupoglazoff et al[4]: notched T waves, also called bifid or humps, were defined as macroscopic bulges or protuberances just beyond the apex or on the descending limb of upright T waves. The bulges or protuberances were called T2 and the immediately preceding T waves were called maximum T1. The U wave was defined as a completely distinct wave after T2 arising either from the isoelectric line or not >0.1 mV greater than the isoelectric line. A subsequent P wave had to arise from the isoelectric line after a preceding U wave, or such an electrocardiogram was excluded to avoid false assessment in case of U- and P-wave fusion. Notched T-wave patterns were assigned to 2 types: type 1 when T1 > T2 and type 2 when T1 < T2 (Figure 1).

The evaluation of T-wave patterns was possible for 95% of electrocardiograms, and interobserver concordance was 100%. All the investigated patients reached

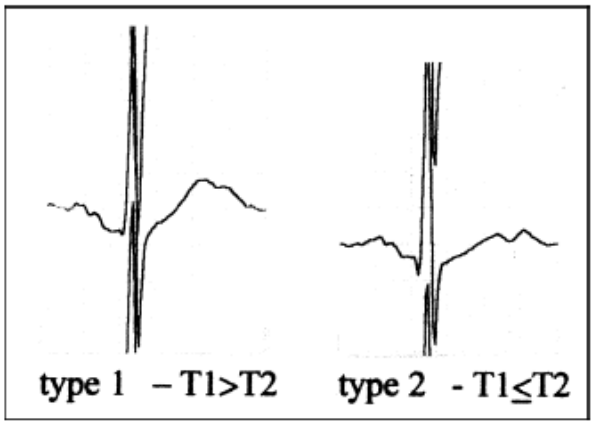

FIGURE 1. Classification of T-wave morphology was adapted from Lehmann et al[3] and Lupoglazoff et al[4]: notched T waves, also called bifid or humps, were defined as bulges or protuberances just beyond the apex or on the descending limb of upright T waves. The bulges or protuberances were called T2, and the immediately preceding T waves were called maximum T1. The notched T-wave patterns were assigned to 2 types: type 1 when T1 > T2 and type 2 when T1 < T2.

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TABLE 1 Clinical Characteristics of Study Subjects (n = 18)

Characteristic	LQT1	LQT2	Noncarriers
Age (yrs)	35 ± 17	51 ± 16	38 ± 19
Men/women	2/4	1/2	4/5
Baseline QTc (s)	0.48 ± 0.05*,†	0.46 ± 0.036*,†	0.42 ± 0.024†
Syncope or cardiac arrest	3	1	0
β-blocker therapy	1	0	0
Number of mutations	2	1	0

*LQT1 compared with LQT2 was not different.
†p <0.01 for patients with LQT1s compared with noncarriers.

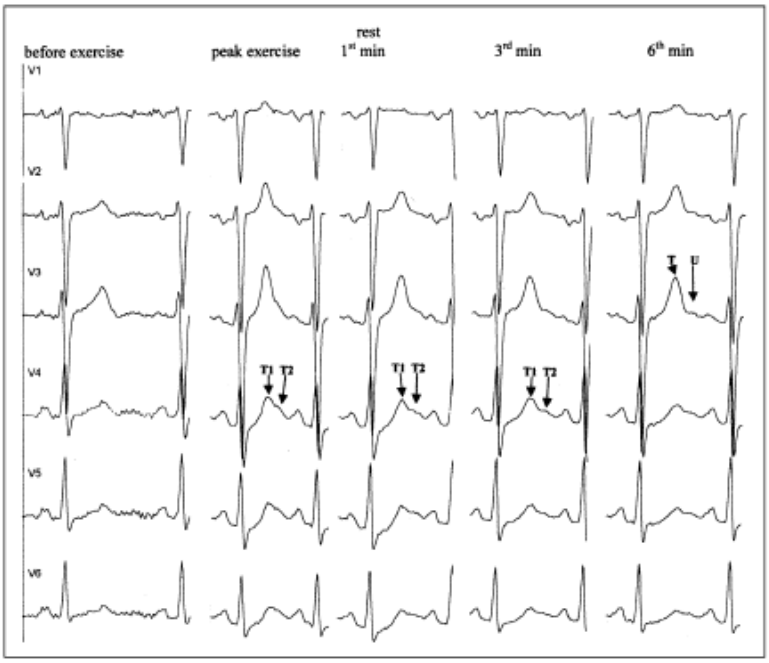

FIGURE 2. The occurrence of a broad-based type 1 notched T wave at peak exercise in a healthy member of a family with LQT1. During 6 minutes of rest, the notched T wave divided into distinctly separated T and U waves (electrocardiographic sweep 50 mm/s, amplitude 20 mm/mV).

the heart rate estimated for submaximal workload according to age and gender.

In the LQT1 families, type 1 notched T waves were observed in 3 members of family 1 and in 1 member of family 2; the finding had zero specificity for the identification of a mutation carrier. The notched T waves occurred at peak exercise, and while assessing the end of T waves as the return to the isoelectric line, the corrected QT intervals reached marked pathologic values. Then, during 6 minutes of rest, these complex forms of T waves divided into distinctly separate T and U waves, and corrected QT interval values were fairly in the physiologic range in all 4 patients (Figure 2).

We hypothesized that in these cases, type 1 notched T waves originated from a fusion of T and U waves at peak exercise. Therefore, we tried to extrapolate the nadir between T and U waves from the sixth minute of rest retrospectively back to the peak exercise electrocardiogram. Using this point as the end of the T wave gave us physiologic values of corrected QT interval in all 4 patients in all the assessed electrocardiographic recordings. Remarkably, as mentioned previously, none of them was a mutation carrier. In affected members of the 2 LQT1 families, no notched T waves occurred.

In LQT2 family 3, in 2 of 3 mutation carriers, type 1 notched T waves occurred at peak exercise. During 6 minutes of rest, they evolved type 2 notched T waves (Figure 3 and Table 2).

In the control group of 18 age- and gender-matched healthy patients, no notched T waves were observed during exercise testing.

• • •

In 6 of 18 members (affected and noncarriers) of 3 families with LQTIS, we observed notched T waves in some phase of exercise testing. In the first minute of rest, all 6 of them presented with type 1 notched T waves. Then, during 6 minutes of rest, these complex T-wave forms evolved into type 2 notched T waves in 2 patients. These were LQT2 mutation carriers. In contrast, in the other 4 patients, the broad-based, type 1 notched T waves divided into distinctly separate T and U waves during 6 minutes of rest. None of these 4 patients was a mutation carrier. Type 1 notched T waves are prevalent but do not seem diagnostically specific. Type 2 notched T waves appear to be specific for LQT2 gene carriers. These observations provide preliminary data to further the understanding of gene-mediated changes on electrocardiographic repolarization parameters.

In LQTIS, in addition to QT interval prolongation, a notched T wave constitutes 1 of the most striking electrocardiographic abnormalities. Studies with Holter monitoring have shown that notched T waves can be observed in 80% of patients with LQT2. This pattern is less common in patients with LQT1 and LQT3. It was also found in 4% of healthy patients in control groups.[1,4] Good specificity of type 2 notched T waves for LQT2 was also observed in our study. In contrast, it is not clear why noncarriers had type 1 notched T waves (4 of 14 patients), whereas there was none in the control subjects. Our observations suggest that these complex forms of T waves can originate from T- and U-wave fusion. The careful observation of T-wave pattern changes over time may help avoid the incorrect diagnosis of LQTIS. It must be stressed that with this sample size, the study is underpowered to

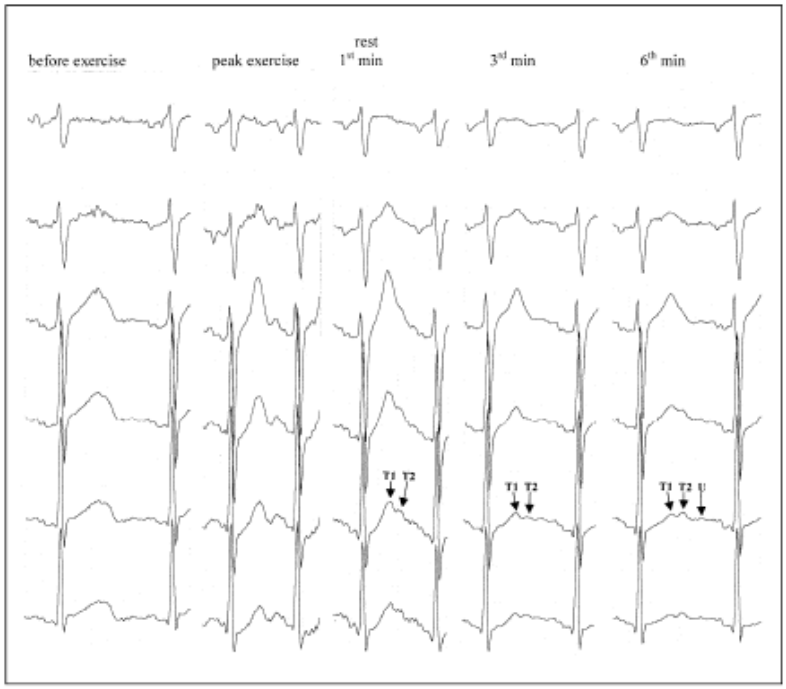

FIGURE 3. The occurrence of a type 1 notched T wave in an LQT2 mutation carrier immediately after the cessation of exercise. During 6 minutes of rest, the T wave evolved in type 2 notched T waves (electrocardiographic sweep 50 mm/s, amplitude 20 mm/mV).

TABLE 2 Notched T-wave Type Occurence in Particular Phase of Exercise Test

	Rest		Peak Exercise		Rest: First Minute		Rest: Sixth Minute	
	Type 1 NT	Type 2 NT	Type 1 NT	Type 2 NT	Type 1 NT	Type 2 NT	Type 1 NT	Type 2 NT
KCNQ1 mutation	0	0	0	0	0	0	0	0
HERG mutation	0	0	0	0	2	0	0	2
Noncarriers	0	0	4	0	4	0	0	0

Type 1 NT = type 1 notched T wave; type 2 NT = type 2 notched T wave.

adequately account for a type II error. The presented data must be considered as preliminary observations.

Although mutational analysis is not yet widely accessible, various clinical investigations have been used for establishing a diagnosis of LQTIS and for risk stratification. This approach enables a clinician to decide on the proper therapy for a particular patient before the results of mutational analysis are available. The congenital forms of LQTIS have been shown to be exquisitely sensitive to increased sympathetic nervous system activity or to exogenously administered catecholamines.[5] Therefore, electrocardiographic recording is particularly helpful in different adrenergic states and is most easily obtained by exercise testing. QT interval prolongation is often more pronounced at peak exercise or immediately after its cessation.[6] T-wave pattern assessment is the next logical step.

The cellular basis for complex T waves has been demonstrated by experimental studies with wedge preparations used for pharmacologic models of LQTIS.[7] In these cases, voltage gradients between 3 different layers of the ventricular wall are responsible for the T-wave pattern. It has been shown that exercise can amplify the notched T-wave morphology in patients with LQTIS, especially those with LQT2,[8] probably because of the increase in the transmural dispersion of repolarization. This phenomenon has not been studied yet in an experimental LQT2 model. Even fewer data are available for the occurrence and explanation of exercise-induced notched T waves in healthy patients. Besides the explanation involving the fusion of T and U waves we have proposed, the notching could be a kind of exercise-dependent phenomenon. Nevertheless, to date, there are no provocative maneuvers to distinguish T- and U-wave fusion from exercise-induced notching.

One hundred years after U waves were described by Einthoven,[9] their origin is still debated.[10] The most popular theory states that U waves are caused by the delayed repolarization of the His-Purkinje system.[11] However, the small mass of the specialized conduction system may be insufficient to generate U waves. Nevertheless, for physiologic U waves, it seems to be the most plausible hypothesis. Under pathologic conditions of acquired or congenital LQTIS, the so-called M cells may be responsible for the inscription of U waves.[12]

The mechanisms underlying T- and U-wave patterns and QT interval duration are complex and incompletely understood. Different channels, regulatory processes, autonomic influences, serum electrolyte levels, and regional differences of ion channel expression in myocardium are likely involved. A differential response of QT and QU intervals to adrenergic stimulation has been described in patients with LQT1.[13] Our observation of a fusion of T and U waves at peak exercise suggests that structures responsible for these electrocardiographic features probably also respond differently to adrenergic stimulation under physiologic conditions.

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Andrsova I, Novotny T, Kadlecova J, Bittnerova A, Vit P, Florianova A, Sisakova M, Gaillyova R, Manouskova L, Spinar J. Clinical characteristics of 30 Czech families with long QT syndrome and KCNQ1 and KCNH2 gene mutations: Importance of exercise testing. J Electrocardiol 2012;45:746-751.

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Jedná se o první systematický popis souboru pacientů z České republiky s LQTS s diagnózou potvrzenou nálezem mutace. Některé z mutací nebyly dosud v literatuře popsány. Soubor je podrobně charakterizován klinicky, je dokladován přínos ergometrického vyšetření.

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Clinical characteristics of 30 Czech families with long QT syndrome and *KCNQ1* and *KCNH2* gene mutations: importance of exercise testing☆,☆☆

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Abstract

Background: Classic symptoms of long QT syndrome (LQTS) include prolongation of QT interval on electrocardiograph, syncope, and cardiac arrest due to a distinctive form of polymorphic ventricular tachycardia, known as Torsade de Pointes. We assessed occurrence of LQTS signs in individuals from 30 Czech families with mutations in *KCNQ1* and *KCNH2* genes.

Methods and Results: One hundred five individuals from 30 Czech families with LQTS were genotyped for *KCNQ1* and *KCNH2*. The occurrence of typical LQTS signs (pathologic prolongation of QT interval; syncope; cardiac arrest; Torsade de Pointes) was clinically assessed by exercise test with QT interval analysis. Family history of sudden cardiac death was taken. Statistical analysis was performed to determine correlation of clinical results and mutation status. *KCNQ1* gene mutations were found in 23 families, and *KCNH2* gene mutations in eight families. Only 46 (70%) of the 66 mutation carriers had at least two of the typical LQTS signs. The others were minimally or asymptomatic. From 39 noncarrier individuals, only 1 fulfilled the clinical criteria of LQTS diagnosis, another 4 had an intermediate probability of diagnosis. The exercise test had 92% sensitivity and 93% specificity for LQTS diagnosis.

Conclusions: Incidence of classical signs of LQTS was not high in Czech carriers of *KCNQ1* and *KCNH2* mutations. Therefore, proper diagnosis relies on detection of symptoms at presentation. The exercise test may be beneficial owing to its high sensitivity and specificity for LQTS diagnosis.

Keywords: Exercise test; LQT syndrome; Mutation; Sudden cardiac death; Torsades de Pointes

Introduction

Long QT syndrome (LQTS) is characterized by an abnormality in myocardial repolarization that leads to typical changes on surface electrocardiogram (ECG), including prolongation of the QT interval, morphological changes of T waves, and appearance of the Torsades de Pointes (TdP).[1] The global prevalence of LQTS has been estimated at 1:2500 but is believed to be higher due to missed diagnosis.[2]

Recent advances in genotyping technologies have led to the recognition of several genes associated with LQTS. Not surprisingly, genes encoding myocardial ion channels have been implicated in LQTS family linkage analysis studies.[3] The *KCNQ1* and *KCNH2* genes, encoding subunits of potassium channels, are among the most frequently associated with LQTS. The aim of this study was to assess genotype–phenotype correlation in 30 Czech families with history of LQTS and mutations in *KCNQ1* and *KCNH2* genes and to evaluate exercise test contribution for the diagnosis of LQTS.

Methods

Study participants

A total of 105 members from 30 families with occurrence of LQTS were included consecutively into the study. All

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individuals underwent physical examination, ECG, and exercise test. The probability of LQTS diagnosis was established using the revised criteria suggested by Schwartz et al.[4] Informed consent was obtained from all participants and blood samples were taken for DNA isolation.

Exercise test

All the individuals were examined by bicycle ergometry to obtain ECG recordings at different adrenergic states. A 12-lead ECG with Mason-Likar modification was used (Marquette-Hellige, CardioSys version 2.5). The initial stress was set to 0.5 W/kg and increased by 0.5 W/kg every 3 minutes.

All ECGs were recorded as paper printings at the speed of 50 mm/s and voltage of 20 mm/mV. QT and R-R intervals were manually measured by 2 blinded investigators for the periods of rest and for each of the first 6 minutes of the recovery period. In most cases the QT interval was measured in lead V_5, the other leads were used only when the end of T wave could not be discriminate in this lead. The QT intervals were corrected for heart rate using Bazett's formula: QTc = (QT/RR$^{1/2}$ measured in seconds).[5]

Mutation analysis

Genomic DNA was isolated from the venous blood samples of each study participant. The purified DNA was applied as template to polymerase chain reaction with exon-specific primers targeting the *KCNQ1* and *KCNH2* genes. The polymerase chain reaction products were screened for the presence of mutation by the single-strand conformational polymorphism method and temperature gradient gel electrophoresis.[6] Exons were sequenced on an ABI PRISM 310 (Applied Biosystems, Foster City, CA, USA). The detailed methodology for this procedure, including exon-specific primer sequences, has been described elsewhere.[7]

Statistical analysis

Parametric data were analyzed by the *F* test for variance and Student *t* test. Nonparametric data were analyzed by the Mann-Whitney *U* and Fisher exact tests.

Results

KCNQ1 gene mutations

KCNQ1 gene mutations (LQTS1) were identified in 22 families (Table 1). Forty-nine individuals were mutation carriers (30 women; 19 men; mean age: 36,6 ± 16,1 years), and 30 had no mutation (21 women; 9 men; mean age: 40,4 ± 11,9 years). Homozygous mutations of the *KCNQ1* gene were found in 2 families, one of which was consanguineous. Both of these familial cases had been described in detail previously.[19,20] Six novel mutations were detected, and none of these was present in the group of healthy individuals (n = 90) with no previous or current symptoms of LQTS. In 1 case yet undescribed, DNA mutation (c.1772G>C) resulted in amino acid change published previously (pR591C).[21]

The previously established LQTS mutation, T309I (C926T), of the *KCNQ1* gene was present in 5 families. It is located in the pore region and its estimated predictive value to be a pathologic mutation is 96% according to Kapa et al.[22]

Table 1
List of mutations.

Gene	Exon	Region	Nucleotide change	Amino acid change	References
KCNQ1	1	N-term	c.453_454insCC	p.P151fsX14	-
	3	S2-S3	c.569G>T	p.R190L†	[8]
	4	S4	c.674C>T	p.S225L	[9]
	6	S5	c.805_819del	p.269_273del	-
	6	Pore	c.916G>C	p.G306R	[8,10]
	7	Pore	c.926C>T	p.T309I*,‡	[8,11]
	7	Pore	c.935C>T	p.T312I	[8,10]
	7	Pore	c.940G>A	p.G314S	[8,12]
	7	S6	c.973G>A	p.G325R	[8,13]
	7	S6	c.1048G>C	p.G350R	[14]
	13	C-term	c.1645_1665del	p.M549_H555del	-
	14	C-term	c.1686G>C	p.R562S	-
	15	C-term	c.1760C>T	p.T587M	[8,15]
	15	C-term	c.1772G>A	p.R591H	[8,7]
	15	C-term	c.1772G>C	p.R591C	-
	16	C-term	c.1831G>A	p.D611N	[8]
	16	C-term	c.1893insC	p.P631fsX650†	[7]
KCNH2	4	N-term	c.815C>T	p.A228V	-
	6	S1-S2	c.1342G>A	p.A448T	-
	7	S4	c.1600C>T	p.R534C	[15]
	7	S5	c.1714G>A	p.G572S	[16]
	7	Pore	c.1894C>T	p.P632S	[17]
	7	S6	c.1919_1921delTCT	p.P640del	-
	13	C-term	c.3131G>T	p.R1047L‡	[18]
	14	C-term	c.3161delG	p.T1054TfsX2	-

* Mutation found in 5 unrelated families.
† Homozygous mutations (2 families).
‡ Compound heterozygosity mutations (1 family).

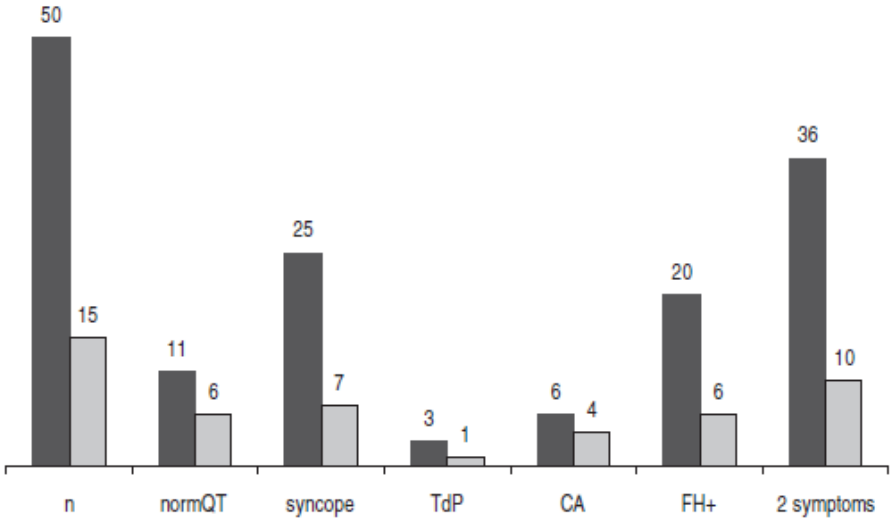

Fig. 1. Incidence of symptoms at initial examination in *KCNQ1* and *KCNH2* mutation carriers. Total num: number of investigated patients; norm QT: number of patients with normal Qtc; TdP: Torsade de Pointes; FH+: number of patients with sudden cardiac death in family history; CA: number of patients who had cardiac arrest; 2 symptoms: patients with two or more LQTS symptoms at initial examination. The dark gray color represents the LQT1 patients and light gray the LQT2 patients.

KCNH2 gene mutation

The mutations of *KCNH2* gene (LQTS2) were found in 7 families (Table 1). We detected 15 mutation carriers (12 women, 3 men, mean age 38,9 ± 15,7). In 8 family members, the mutation of *KCNH2* gene was not found (6 women, 2 men; mean age, 48.7 ± 17.9 years). One of the *KCNH2* gene mutations was novel.

Compound heterozygosity

In one family of 4 members, the compound heterozygosity of *KCNQ1* and *KCNH2* mutations was present (*KCNQ1:* p.[Thr309ILe], *KCNH2:* p.[Arg1047Leu]). We detected 2 mutation carriers—proband and his father, mother and older brother were healthy.

Baseline clinical findings in families with KCNQ1 gene mutations

The mean QTc at rest was longer in the mutation carriers than in the non-mutation carrier family members (479 ± 57 vs 413 ± 31 milliseconds, $P < .001$). Nevertheless, 11 pathologic mutation carriers (22%) had physiological QTc at baseline assessment, and only 36 patients (73%) had at least 2 typical signs of LQTS. Fig. 1 summarizes the incidence of symptoms in mutation carriers. Moreover, the mean QTc at rest was also longer in symptomatic carriers than in asymptomatic carriers (510 ± 72 vs 459 ± 32 milliseconds, $P < .001$).

According to Schwartz scores, only 32 mutation carriers (65%) had a high probability of diagnosis, while the other mutation carriers were minimally symptomatic or asymptomatic (Fig. 2A). According to clinical symptoms (QTc 480 milliseconds and sudden cardiac death in family history), only 1 woman among the non–mutation-carrier family members had an intermediate probability of LQTS diagnosis, while another 2 were minimally symptomatic (Fig. 2B).

Baseline clinical findings in families with KCNH2 gene mutations

The mean QTc at rest was longer in the mutation carriers than in the non-mutation carrier family members (454 ± 47 vs 414 ± 33 milliseconds, $P < .001$). Difference in QTc value between symptomatic and asymptomatic mutation carries was not statistically significant (482 ± 56 vs 440 ± 36 milliseconds). Six mutation carriers (40%) had normal QTc value at start. Incidence of symptoms in mutation carriers is summarized in Fig. 1. The probability of LQTS diagnosis according to Schwartz score in mutation carriers is summarized in Fig. 2A and in mutation noncarriers in Fig. 2B.

Baseline clinical findings in families with compound heterozygosity

Both of the mutation carriers were asymptomatic. The proband was investigated due to palpitation and detection of QTc prolongation during the 24 hours ECG monitoring. The rest QTc value was 440 milliseconds, the longest QTc from 24 hours ECG monitoring was 507 milliseconds. The other mutation carrier, the father, had QTc of 480 milliseconds initially.

Bicycle ergometry findings in families with KCNQ1 gene mutations

The patients completed the bicycle ergometer test. It was possible to assess 95% of the resultant ECG prints, but 5% were excluded due to obvious artifactual data.

Forty-eight (96%) mutation carriers were found to have further pathologic prolongation of QTc during exercise test, which increased the numerical values of their Schwartz scores. Two non-carrier family members had pathologic prolongation of QTc, as well. The detected changes in QT interval are summarized in Tables 2 and 3.

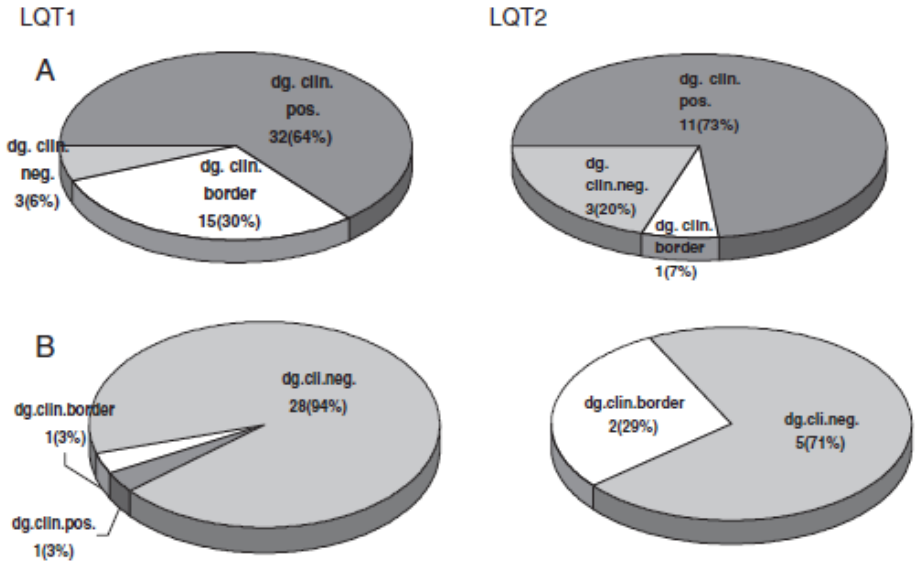

Fig. 2. A. LQTS diagnosis probability according to clinical symptoms at initial examination in pathologic mutation carriers, B. LQTS diagnosis probability according to clinical symptoms at initial examination in non-mutation carriers. (dg clin +: high probability of diagnosis according to clinical signs – Schwartz score ≥3.5; dg clin neg: low probability of diagnosis according to clinical signs Schwartz score ≤ 1; dg clin border: intermediate probability of diagnosis according to clinical signs - Schwartz score 1.5-3).

Bicycle ergometry findings in families with KCNH2 gene mutations

The pathologic prolongation of QTc interval during the stress test was detected in 12 (80%) mutation carriers. In one non carrier family member pathologic prolongation was observed too. The detected changes in QT interval are summarized in Tables 2 and 3.

Bicycle ergometry findings in families with compound heterozygosity

The proband did not undergo ergometry because of low age and low body height. The pathologic prolongation of QTc interval was observed only in the father (510 milliseconds). The non carrier members—the mother and the older brother—had physiologic QTc values.

Discussion

This study was designed to determine the genotype-phenotype correlation in a small group of Czech patients with LQTS that was confirmed by mutational analysis. It represents the first systematic description of LQTS individuals in the central European region.

Our data supported the previously published findings that indicate a large proportion of LQTS mutation carriers are minimally symptomatic or asymptomatic.[23,24] Therefore, careful clinical investigations and physician awareness are important to identify LQTS patients and reduced missed diagnosis.[25-27] The bicycle ergometer test is a convenient, noninvasive method used to evaluate the QT interval; because it induces the adrenergic condition naturally and modern ergometers can produce high quality ECG recordings, it is considered acceptable by both patients and treating physicians/institutions.[28] Another exercise equipment-based method that is available is the treadmill; however, this test produces more ECG artifacts due to upper body movements.

Fifty-nine (89%) mutation carriers produced further pathologic prolongation of QTc during the exercise test in this study. This stress test induced symptoms that increased the numerical value of the individual's Schwartz score. Therefore, despite the known technical limitations of the stress test, including determination of the T wave end and

Table 2
QTc values in mutation carriers *vs.* non-mutation family members.

LQT1	Mutation carriers	Non-mutation family members	*P*
	n = 49	n = 39	
QTc rest	0.479 ± 0.057	0.413 ± 0.031	<.001
QTc exercise	0.534 ± 0.057	0.426 ± 0.020	<.001
LQT2	n = 5	n = 10	
QTc rest	0.454 ± 0.047	0.414 ± 0.033	<.001
QTc exercise	0.484 ± 0.045	0.427 ± 0.028	<.001

Data are presented as average ± SD.

Table 3
QTc values in symptomatic *vs.* asymptomatic mutation carriers.

LQT1	Symptomatic mutation carriers	Asymptomatic mutation carriers	*P*
	n = 20	n = 30	
QTc rest	0.504 ± 0.069	0.455 ± 0.34	<.001
QTc exercise	0.549 ± 0.062	0.504 ± 0.047	<.005
LQT2	n = 5	n = 10	
QTc rest	0.510 ± 0.072	0.459 ± 0.032	.08
QTc exercise	0.557 ± 0.066	0.516 ± 0.044	.04

Data are presented as average ± SD.

methodology of QT interval correction, it was considered to significantly contribute to establishment of clinical diagnosis of LQTS. Moreover, in our study the stress test helped to identify individuals with increased risk of life-threatening arrhythmias.

In Czech patients mutations of *KCNQ1* gene were 3 times more frequent than mutations of *KCNH2* gene. On the contrary the Danish registry has the opposite distribution.[29] Other groups report balanced proportion of both genes mutations.[22] If these are truly regional differences or they are rather caused by low numbers of investigated patients remains to be established.

Original mutations were found in almost all families evaluated in our study. The exception was the T309I mutation of the *KCNQ1* gene, which has been previously identified in other LQTS populations. This variant was present in 5 unrelated families in our study and it segregated with the LQTS phenotype. While no functional studies have yet been published for this particular variant, it is known to be located in the pore region, where it is very likely to affect pore function and LQTS. Moreover, the estimated predictive value of this mutation was previously reported as 96%.[22] Further investigations should be conducted, however, to determine whether T309I is a founder mutation in ethnic-specific population.

There are several limitations to be considered. The technical problems were cited above for the ergometry test. QTc measurements were made using Bazett's formula. This formula has wide acceptance and use, but it can overcorrect and undercorrect for heart rates greater than 100 and 60 bpm, respectively.[30] Other limitations of our study exist and should be considered when interpreting our results. For example, small group of patients especially in the LQT2 families which affected the statistical results. Also, none of the novel mutations identified in our set of Czech LQTS families have been investigated in functional studies. Nevertheless, the phenotype-genotype correlations were clear in most families, and we believe future functional studies of mutated channels will provide insights into their functional effects in LQTS.

Conclusion

The incidence of classical signs of LQTS was not high in the Czech *KCNQ1* and *KCNH2* mutation carriers. The exercise test, using a bicycle ergometer, has high sensitivity and specificity and may be a feasible and effective method of diagnosis. Mutation analysis may still prove useful as an additional means of risk stratification.

Author contributions

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Critical version of article: Tomas Novotny, Alena Florianova.

Data collection: Alena Florianova, Lenka Manouskova, Irena Andrsova, Pavel Vit.

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2.1.4. Molekulárně genetická diagnostika LQTS – komentář k současným poznatkům

V poslední dekádě 20. století došlo k přelomovému pokroku v chápání podstaty LQTS na molekulárně genetické úrovni. V rychlém sledu bylo identifikováno několik genů, jejichž mutace jsou zodpovědné za rozvoj onemocnění. Molekulárně genetické vyšetření bylo prezentováno jako úhelný kámen diagnostiky a řada pracovišť po celém světě prováděla mutační analýzy v různých souborech pacientů s cílem objevení dalších souvisejících genů, ať už metodami vazebné analýzy či kandidátních genů. S přibývajícími daty se však dostavilo určité vystřízlivění. Dnes je diagnóza postavena na klinických známkách a rutinní provádění mutační analýzy bylo expertní skupinou odborných arytmologických společností (22) u LQTS doporučeno v následujících případech:

- pacient s klinickým podezřením na LQTS (patologická hodnota QT_c + symptomy)
- asymptomatický jedinec s QT_c nad 480 ms u dětí, nad 500 ms u dospělých
- příbuzný LQTS pacienta, u kterého již byla nalezena mutace

Raudenská M, Bittnerová A, <u>Novotný T</u>, Floriánová A, Chroust K, Gaillyová R, Semrád B, Kadlecová J, Šišáková M, Toman O, Špinar J. Mutation analysis of candidate genes SCN1B, KCND3 and ANK2 in patients with clinical diagnosis of long QT syndrome. Physiol Res 2008;57(6):857-862.

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Soubor vyšetřených sestával z 12 nepříbuzných jedinců s klinickou diagnózou LQTS, u nichž nebyly nalezeny mutace v 5 nejčastěji postižených genech. Práce obsahuje jejich podrobnou klinickou charakteristiku a dále výsledky mutační analýzy dalších kandidátních genů kódujících různé proteiny iontových kanálů myokardu. V žádném z nich nebyly nalezeny kódující varianty, které by mohly být příčinou LQTS.

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Mutation Analysis of Candidate Genes *SCN1B, KCND3* and *ANK2* in Patients with Clinical Diagnosis of Long QT Syndrome

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Summary

The long QT syndrome (LQTS) is a monogenic disorder characterized by prolongation of the QT interval on electrocardiogram and syncope or sudden death caused by polymorphic ventricular tachycardia (torsades de pointes). In general, mutations in cardiac ion channel genes (*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*) have been identified as a cause for LQTS. About 50-60 % of LQTS patients have an identifiable LQTS causing mutation in one of mentioned genes. In a group of 12 LQTS patients with no identified mutations in these genes we have tested a hypothesis that other candidate genes could be involved in LQTS pathophysiology. *SCN1B* and *KCND3* genes encode ion channel proteins, *ANK2* gene encodes cytoskeletal protein interacting with ion channels. To screen coding regions of genes *SCN1B, KCND3,* and 10 exons of *ANK2* following methods were used: PCR, SSCP, and DNA sequencing. Five polymorphisms were found in screened candidate genes, 2 polymorphisms in *KCND3* and 3 in *SCN1B*. None of found polymorphisms has coding effect nor is located close to splice sites or has any similarity to known splicing enhancer motifs. Polymorphism G246T in *SCN1B* is a novel one. No mutation directly causing LQTS was found. Molecular mechanism of LQTS genesis in these patients remains unclear.

Key words

ANK2 • Candidate genes • KCND3 • Long QT syndrome • SCN1B

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Introduction

The long QT syndrome (LQTS) is a monogenic disorder characterized by prolongation of the QT interval on electrocardiogram and syncope or sudden death caused by polymorphic ventricular tachycardia (torsades de pointes).

In general, mutations in cardiac ion channel genes (*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*) have been identified as a cause for LQTS (Splawski *et al.* 2000). Recently, mutation in cytoskeletal protein gene *ANK2* has also been linked to LQTS in one French family (Mohler *et al.* 2003, 2004). About 50-60 % of LQTS patients have an identifiable LQTS-causing mutation in one of the 5 most prevalent cardiac channel genes (Tester *et al.* 2005). In the others the disease mechanism remains unclear. The aim of this study is to test the hypothesis that other cardiac ion channel genes could be involved in LQTS pathophysiology: *SCN1B* gene encodes β1 subunit of cardiac Na^+ channel and plays an important role in channel inactivation (Wallace *et al.* 2002). *KCND3* is a gene that encodes the K^+ channel that underlies the potassium transient outward current I_{to} in the human ventricle (Dixon *et al.* 1996). Targeted mutational analysis of *ANK2* was also completed.

Methods

Group of patients

The group of patients was recruited from

Table 1. Clinical characteristics of study subjects.

Patient	Age	Sex	QTc (ms)	Syncope	Torsades de pointes	LQTS in family	Diagnostic score (points)
IB	64	F	0.48	0	0	+	4
MK	62	F	0.5	+	+	+	7
MI	42	F	0.48	+	0	+	5
LP	13	M	0.47	0	0	+	3
MZ	25	F	0.5	+	0	+	5
MK	25	M	0.47	+	0	+	4
PD	25	F	0.5	+	0	0	4
MD	58	F	0.54	+	+	0	6
JJ	46	M	0.47	0	0	+	3
AL	20	F	0.49	+	0	0	4
MS	16	F	0.48	+	+	0	5
ŠK	17	F	0.48	+	0	+	5

Sex: M – male, F – female, QTc – QT interval corrected to heart rate according to Bazett formula ($QTc=QT/RR^{1/2}$), diagnostic scoring: 2-3 points – intermediate probability of LQTS, ≥4 points – high probability of LQTS.

25 unrelated individuals who were consecutively referred to the Department of Internal Medicine and Cardiology with suspicion on LQTS. They were examined clinically including bicycle ergometry. In all of them intermediate or high probability of LQTS was present based on diagnostic score (Schwartz *et al.* 1993). In this scoring system various point values are assigned to various symptoms, a value of 2-3 indicates intermediate probability and ≥ 4 points indicates high probability of LQTS diagnosis.

Informed consent was obtained from all the individuals and peripheral blood samples were taken. Mutational analysis of *KCNQ1, KCNH2, SCN5A, KCNE1* and *KCNE2* genes was performed by methods published elsewhere (Splawski *et al.* 2000). In 11 patients the KCNQ1 gene mutations and in 2 patients the KCNH2 gene mutations were present. In the other 12 patients no mutation in the above mentioned LQTS related genes was found. These 12 individuals were subjects of this study – mutational analysis was extended to following genes: *SCN1B, KCND3* and exons 36-37 and 39-46 of *ANK2*. The brain-specific exon 38 was not analyzed in this study. Clinical characteristics of study subjects are summarized in Table 1.

Genomic DNA Extraction and PCR

Genomic DNA was extracted from samples of peripheral blood according to the standard protocol using DNA BloodSpin Kit and the standard chloroform/ethanol method. Eleven oligonucleotide primer pairs were used to amplify coding area of *KCND3*, as proposed by Postma *et al.* (2000), only the fifth segment of the first coding exon had different reverse primer (GGT CAT CCA GCT GCC CTC CAA CCT), which agrees with sequence of *KCND3* in NCBI database (accession number NT 019273). Ten primer pairs were disposed to amplify chosen exons of *ANK2* (from 36 to 46 except 38). These primers were taken from Mohler *et al.* (2003). For the PCR amplification of SCN1B gene coding and UTR region were suggested primers using Primer3 (Rozen and Skaletsky 2000), (Table 2). Sequences of these primers agree with sequence of *SCN1B* in Ensembl database (© 2006 WTSI / EBI, ENSG00000105711).

SSCP analysis

For the analysis of *KCND3* and *ANK2* 3µl aliquots of the amplified sample were mixed with 5 µl of bromphenol blue loading dye, for analysis of *SCN1B* 1 µl aliquots of the amplified sample were mixed with 3 µl of bromphenol blue loading dye and 6 µl of destilated water. Samples were subsequently denatured by heating at 94 °C for 5 min, and placed into cold water to avoid renaturation. Then the samples were loaded on 9 % (*KCND3*), 11 % (*ANK2*) and 10 % (*SCN1B*) non-denaturing PAA gel. Electrophoresis was performed at 200 V and 10 °C for 3 h (*KCND3* and *ANK2*) and at 120 V and 18 °C for 12 h (all exons of *SCN1B* except exon 3). Exon 3 was performed at 10 °C.

Table 2. Oligonucleotide primers for detection of mutations in *SCN1B*.

Exon	Size, bp	Forward Primer Sequence	Reverse Primer Sequence
1.1	250 bp	5'-GGTGCACCTAGCGGATGT-3'	5'-TATTAATAGCGGGGCGAGAG-3'
1.2	284 bp	5'-GCTCCCGGGGACATTCTA-3'	5'-AACTTCTGAAGCTGACTTGG-3'
2	186 bp	5'-CTGTCCCCACAGTGTCCT-3'	5'-CGCACACCTTGACAAACT-3'
3	266 bp	5'-CTGGCTACCCCTAGATCCT-3'	5'-ACCCGACTCACCTTTGTC-3'
4	200 bp	5'-CTGGGCTACCCCCTTAACC-3'	5'-ACCTCCCAGCCACCCTACT-3'
5	196 bp	5'-GGTCTGATGATGGGGTCACT-3'	5'-GCAAGAGAGGGGGAATTAGG-3'
6.1	267 bp	5'-CCTAATTCCCCCTCTCTTGC-3'	5'-AGCGGCAGTATTGCTTTACC-3'
6.2	289 bp	5'-CACTTTCGCCTCCTCCAG-3'	5'-CGGAGTGGGTCCCAGAAC-3'
6.3	275 bp	5'-GATGATGGGCTGGAGCAG-3'	5'-GGGGCTCACAATCGAAACTA-3'

Table 3. Single nucleotide polymorphisms in *SCN1B*, *KCND3* and *ANK2* genes.

Patient	*SCN1B*	*KCND3*	*ANK2*
I	non	non	non
II	non	C264T/non	non
III	IVS+15 G>T/non = G246T/non	non	non
IV	IVS+15 G>T/non = G246T/non	C264T/non	non
V	non	non	non
VI	non	non	non
VII	non	C264T/C264T	non
VIII	non	non	non
IX	non	G669C/non	non
X	non	non	non
XI	T9204C/T9204C, A9248C/A9248C	non	non
XII	non	non	non

DNA sequencing

Genomic DNA obtained from 12 unrelated LQTS patients was screened for mutations in the coding regions of the genes *SCN1B*, *KCND3* and a part of the gene *ANK2* using SSCP analysis. If we have found three or four single strand bands in the SSCP patterns then we have done sequencing with forward and reverse sequencing primers. For purification of the amplified samples MinElute PCR Purification Kit (QIAGEN) was used. For cycle sequencing we used Big Dye Terminator Kit (Applied Biosystems) and for purification of samples after cycle sequencing DyeEx2.0 Spin Kit (Qiagen). Exons were sequenced by instrument ABI PRISM 310 (Applied Biosystems, USA).

Results

The result of the mutation screening is listed in the Table 3. None of found SNPs led to amino acid changes, were located close to splice sites or had any similarity to known splicing enhancer motifs.

Discussion

About 50-60 % of LQTS patients have an identifiable LQTS-causing mutation in one of the five most prevalent cardiac channel genes. In the others the pathophysiology remains unknown. Possible mechanisms include involvement of other ion channels but also regulatory and other proteins. Recently, possible link to LQTS was found in several new genes: *KCNJ2* mutations in Andersen-Tawil syndrome (Tawil *et al.* 1994, Plaster *et al.* 2001), *CACNA1c* mutation in Timothy syndrome (Splawski *et al.* 2005), *CAV3* and *SCN4B* mutations in some LQTS patients (Vatta *et al.* 2006, Medeiros-Domingo *et al.* 2007). These are either rare complex neurological disorders (prolonged QT interval being not the leading symptom) or there are only anecdotal cases.

Thus it is still discussed if these diseases should be included among LQTS.

In this study we tested the hypothesis of other ion channels involvement. Three candidate genes were chosen for mutational analysis in 12 patients with clinical diagnosis of LQTS. *KCND3* encodes the K^+ channel that underlies the potassium transient outward current I_{to} in the human ventricle (Dixon *et al.* 1996). I_{to} is especially important during the early phase of repolarization, as it sets the plateau voltage of both action potential (Tseng 1999).

In contrast to other genes, *ANK2* does not encode an ion channel but encodes a structural protein called ankyrin B that is most likely implicated in ion channels anchoring to the cellular membrane. A clue to the basis of this variant emerged from a study by Chauhan *et al.* (2000), in which sodium channel activity was altered in mice lacking ankyrin B – not an ion channel protein, but an adaptor protein that associated with cytoskeletal interactions. In this study, the ankyrin B spectrin-binding domain encoded by exons 36 and 37 of *ANK2* and the entire C-terminal domain encoded by exons 39-46 were screened. The brain-specific exon 38 and membrane-binding domain were not analyzed in this study.

SCN1B encodes the voltage-gated Na^+-channel β1 subunit (SCN1B). *SCN1B* is expressed in brain, skeletal muscle and heart. The α subunit alone can display functional channel properties, but requires the β subunits to modulate Na^+-channel inactivation. Mutations in Na^+-channel genes are known to cause paroxysmal excitability phenomena in skeletal muscle (myotonia, periodic paralysis) and heart (long QT syndrome) (Wallace *et al.* 2002). In the mutation database (Stenson *et al.* 2003) only one mutation in the SCN1B gene has been published. It is the substitution mutation C387G (on protein level C121W). This mutation causes a disruption of disulfide bridge and may alter the secondary structure of the extracellular domain (Wallace *et al.* 2002).

In our study, two allelic variants were found in *KCND3*. All variants were single nucleotide polymorphisms (SNPs) in coding regions. None of the coding SNPs led to amino acid changes, was located close to splice sites or had any similarity to known splicing enhancer motifs (Liu *et al.* 1998). All SNPs in *KCND3* were described in normal population too (Frank-Hansen at al. 2005). We assume that all the changes detected in the LQTS patients were normal variants.

No allelic variant has been found in *ANK2*gene. Three allelic variants (all SNPs) were found in *SCN1B* gene, two of them T9204C and A9248C were detected previously (Ensembl database, © 2006 WTSI / EBI, ID:ENSG00000105711), G246T has not been published yet. By the help of ESEfinder Release 2.0 (Cartegni *et al.* 2003) we have found that G246T and T9204C substitution does not have any influence on DNA splicing. The substitution A9248C causes loss of one binding place for SC35 and SRp40 splicing proteins.

The fact that no mutations have been found, can have several reasons. The SSCP methodology can detect 88 and 90 % of mutations (Fodde and Losekoot 1994) and therefore we can not fully exclude the presence of mutations within analyzed genes in these individuals. The SSCP method is sensitive for the detection of point mutations or small deletions, but it can possibly miss large deletions. The SSCP method is the most widely used screening method because of its relative simplicity and low costs. Compared to more advanced methods, such as TmHPLC (Temperature Modulated High-Performance Liquid Chromatography) the SSCP has relatively low sensitivity.

LQTS causing mutations could also present in such regions of ion channel genes which are usually not examined. Alternatively, intronic mutations or mutation in the promoter and regulatory regions could be responsible for the disease symptoms. The possibility of other than ion channel genes being involved in the etiology of the long QT syndrome should not be refused, because of huge genetic heterogeneity of this disorder (see the above mentioned genes with recently discovered possible link to QT interval prolongation). This heterogeneity makes genetic testing much more difficult than in case if a single gene were responsible for the disease.

Molecular mechanism of LQTS genesis in our group of patients remains unclear.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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2.1.5. Polékový syndrom dlouhého QT intervalu

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(publikovaná přehledová práce)

PROARYTMICKÉ ÚČINKY NEKARDIÁLNÍCH LÉKŮ NA PODKLADĚ PRODLOUŽENÍ INTERVALU QT

Tomáš Novotný

Proarytmie – vyvolání nové arytmie nebo zhoršení již přítomné arytmie terapeutickou dávkou léčiva – je již dlouho známa u antiarytmik. Projevuje se v drtivé většině případů prodloužením QT intervalu a komorovou arytmií torsade des pointes, která může pacienta i usmrtit – hovoříme o polékovém syndromu dlouhého QT intervalu. V poslední době byla proarytmie popsána rovněž u celé řady nekardiovaskulárních léků. Výskyt jevu zatím nebyl přesně kvantifikován, mechanizmus zůstává nejasný. Důležitá je zřejmě individuální predispozice způsobená odchylkami ve funkci srdečních iontových kanálů nebo degradačních enzymů léčiv. Článek shrnuje také rizikové faktory proarytmie a na jejich základě uvádí možnosti prevence.
Klíčová slova: gen, proarytmie, QT interval, torsade des pointes

PROARRHYTHMIC EFFECTS OF NONCARDIAC DRUGS

The proarrhythmia fenomenon – an induction of new arrhythmia or worsening of preexisting arrhythmia – is well known in antiarrhythmic drugs for many years. In majority of cases the proarrhythmia presentation consists of QT interval prolongation and ventricular tachycardia torsade des pointes, which can be even cause of death. Recently it has become apparent that proarrhythmia can occur during treatment with a long list of noncardiovascular drugs. Incidence of the phenomenon has not been quantified, the mechanism remains unclear. A factor of particular importance is a particular predisposition of individual patient based on variations in function of cardiac ion channels and drug-metabolizing enzymes. Risk factors of proarrhythmia are rewieved and possibilities for prevention are proposed.
Key words: gene, proarrhythmia, QT interval, torsade des pointes

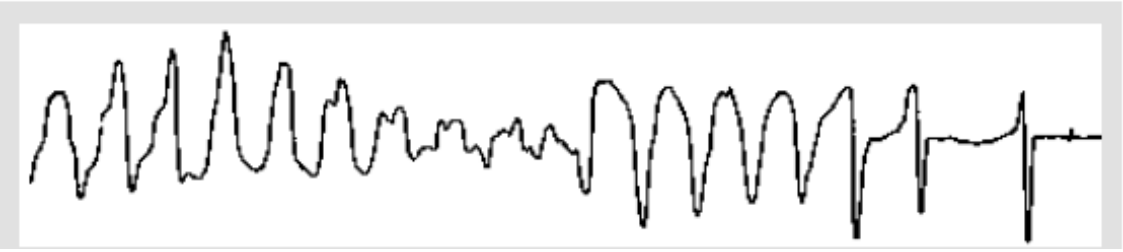
Kardiol. prax 2004; 2 (1): xx-xx

Pod pojmem proarytmie rozumíme vyvolání nové arytmie nebo zhoršení již přítomné arytmie terapeutickou dávkou léčiva (tedy ne předávkováním). Je to fenomén již dlouho známý u antiarytmik. Například až u 4,8 % pacientů léčených d, l – sotalolem byl popsán výskyt polymorfní komorové tachykardie typu „torsade de pointes" (TdP) (**Obrázek 1**)[1]. Tato maligní arytmie je příčinou synkopy nebo může pacienta i usmrtit. Zvýšené riziko maligních arytmií mají především jedinci, u nichž po nasazení preparátu dojde k prodloužení korigovaného QT intervalu povrchového EKG, zejména při prodloužení nad 0,5 sekundy.

Stejné příznaky (tzn. prodloužení QT intervalu, TdP, synkopy, náhlá smrt) charakterizují také onemocnění zvané **kongenitální syndrom dlouhého QT intervalu** (LQTS). Syndrom byl popsán na přelomu 50-tých a 60-tých let[4, 5] a jeho etiologie zůstávala dlouho nejasná. Zásadní průlom znamenal rok 1991, kdy byla prokázána vazba LQTS na 11. chromozom[6]. V následujících letech byl zjištěn vztah i k dalším chromozomům. Dnes tyto syndromy chápeme jako společné fenotypové vyjádření nejméně 9 geneticky odlišných nozologických jednotek. Jejich příčinou jsou v převážné většině případů mutace genů kódujících jednotlivé podjednotky iontových membránových kanálů. Tyto kanály hrají důležitou roli v tvorbě a trvání akčního potenciálu kardiomyocytů. Jejich postižení vede k rozvoji klinického obrazu LQTS. Zevními faktory vyvolaná forma syndromu je označována jako **získaný LQTS**. V tomto článku se zabýváme polékovým LQTS.

Do dnešního dne bylo identifikováno asi 200 chemických sloučenin – farmak (zavedených nebo ve vývoji), které mohou prodloužit QT interval a vyvolat TdP. Přitom v drtivé většině tyto preparáty nemají s kardiologií nic společného a jsou to léky poměrně běžně užívané. V dnešní éře polypragmázie nejsou výjimečné ani kombinace těchto léků, což je faktor výrazně zvyšující nebezpečí proarytmie. Seznam léků s proarytmogenním účinkem je uveden

Obrázek 1 Polymorfní komorová tachykardie typu „torsade des pointes" – typický projev proarytmie.



v **tabulce 1**, která si ale v žádném případě neklade nároky na úplnost. Seznam je totiž neustále doplňován. Aktualizace je možná prostřednictvím internetu, např. na www.torsades.org.

Tabulka 1. Léky prodlužující QT interval

antiarytmika	ajmalin amiodaron bretylium dofetilid disopyramid ibutilid prokainamid propafenon chinidin sotalol
antibiotika, chemoterapeutika, antimykotika	amantadin clarythromycin chloroquin cotrimoxazol erythromycin flukonazol halofantrin itrakonazol ketokonazol pentamidin chinin spiramycin sparfloxacin
antihistaminika	astemizol loratadin terfenadin
psychofarmaka	amitryptilin clomipramin clozapin chlorpromazin citalopram desipramin doxepin droperidol fluphenazin haloperidol imipramin lithium maprotilin mesoridazin nortryptilin perícyclin pimozid prochlorperazin quietapin risperidon sertindol sultoprid thioridazin timiperon trifluoperazon zimeldin ziprasidon
různé	cisaprid indapamid ketanserin probucol sildenafil vasopresin

Výskyt jevu

Proarytmický potenciál jednotlivých léků se určitě liší, ale dostatečně kvantifikován výskyt jevu dosud nebyl. Jako příklad může sloužit výše uvedený sotalol, u něhož byly popsány TdP až u 4,8 % pacientů (t.j. až 1:20) a z nekardiovaskulárních preparátů jsou k dispozici jen odhady, např. 1:120 000 u cisapridu[5]. Nedávno byly publikovány dvě obrovské studie využívající administrativní data (90 000 resp. 480 000 pacientů), které sledovaly výskyt náhlé smrti u pacientů léčených psychofarmaky[6,7]. Obě studie zjistily 2–3x vyšší riziko náhlého úmrtí u psychiatrických pacientů. I když jistě musíme počítat s multifaktoriální etiologií, riziko náhlé smrti vykazovalo závislost na konkrétním preparátu a výši léčebné dávky.

Ještě méně informací máme k dispozici o výskytu prodlužování QT intervalu u konkrétních preparátů. Největší studie věnující se EKG změnám u psychofarmak zahrnuje pouhých 495 pacientů, přičemž u 8 % z nich bylo pozorováno prodloužení QT intervalu[8]. Rozsáhlejší epidemiologické průzkumy nejsou k dispozici. V současné době běží nejméně 1 rozsáhlá studie věnující se tomuto jevu[9].

Mechanizmus jevu

QT interval povrchového EKG odráží trvání akčního potenciálu komorových myocytů, především trvání repolarizace. Je to proces tvořený křehkou rovnováhou mezi celou řadou iontových proudů dovnitř a ven z buňky. Z tohoto hlediska je důležité, že společnou vlastností prakticky všech preparátů prodlužujících QT interval je blokáda draslíkového kanálu I_{Kr} v srdečních myocytech[10]. Proč však přesto k prodloužení QT intervalu a rozvoji maligních arytmií dochází jen u zlomku pacientů?

Zcela zásadním faktorem je zřejmě individuální predispozice konkrétního jedince. V roce 1999 bylo popsáno několik případů „forme fruste" kongenitálního LQTS[11]. V některých rodinách dosahovala penetrance plného vyjádření choroby jen 25 %. Ostatní nositelé patologických mutací genů pro iontové kanály v těchto rodinách zůstávali zcela asymptomatičtí, tedy neměli ani prodloužený QT interval. Normální hodnota QT intervalu tak rozhodně nevylučuje, že konkrétní jedinec není nositelem potenciálně nebezpečné mutace. Možným vysvětlením je **koncepce „repolarizační rezervy"**[12]. Za normálních okolností je totiž mírné zhoršení funkce některého iontového kanálu vyrovnána zvýšenou aktivitou jiných kanálů. Pokud se však takový jedinec dostane do „zátěžové situace" (např. riziková medikace), může být repolarizační rezerva vyčerpána a dochází k prodloužení QT intervalu a zvýšenému riziku maligních arytmií.

Významnou roli hrají jistě i **individuální rozdíly v metabolizmu léčiv**. U člověka se v odbourávání léků uplatňuje více než 30 rodin enzymů a u řady z nich byly popsány genetické varianty, kterou mohou být příčinou výrazných rozdílů ve farmakokinetice i farmakodynamice. Terapeutická hladina stejného léku se tak u dvou jedinců může lišit až 10x při stejném dávkování[13]. Tematika enzymatických systému účastnících se v odbourávání léků je velmi

obsáhlá a není možné se jí v tomto článku více věnovat. Zájemce o další informace odkazuji na citaci 13 (článek volně přístupný na www.nejm.org).

V obou případech se nemusí jednat o vyloženě patologické mutace. Existuje celá řada tzv. běžných polymorfizmů genů kódujících iontové kanály nebo enzymy uplatňující se v metabolizmu léčiv. Určité procento populace je tak pravděpodobně zatíženo skrytým rizikem rozvoje proarytmie. Nicméně k rozvoji proarytmických jevů je v drtivé většině případů nutné spolupůsobení více rizikových faktorů[14].

Rizikové faktory

Rizikové faktory shrnuje **tabulka 2**.

1) **Hraniční nebo dokonce prodloužený QT interval před zahájením léčby** (QT_c = 0,45 s a více) může být fenotypovým projevem výše zmíněných polymorfizmů.

2) Ženy mají obecně delší QT interval, proto je **ženské pohlaví** považováno za rizikový faktor. Zajímavé je, že v experimentu bylo pozorováno snížení exprese repolarizačních kanálů vlivem ženských pohlavních hormonů[15].

3) Osoby **vyššího věku** mají zvýšené riziko patologického prodloužení QT intervalu.

4) Extrémně závažné jsou **lékové interakce**. Celá řada léků je totiž odbourávána prostřednictvím téhož enzymatického systému – cytochromu P450, jiné preparáty vedou k jeho inhibici. Navíc různé skupiny léčiv mohou vykazovat podobný farmakologický účinek – blokádu draslíkových kanálů. Jejich efekt se pak sčítá. Především jsou to preparáty, u nichž bylo pozorováno prodlužování QT intervalu. Cytochrom P450 je ale inhibován např. i šťávou z grepů.

5) **Hypokalémie** prodlužuje trvání repolarizace negativním ovlivněním vodivosti draslíkových repolarizačních kanálů. Příčinou hypokalémie mohou být hormonální poruchy, průjmová onemocnění nebo vedlejší účinky některých léků (nejčastěji diuretika u kardiaků a hypertoniků). Za hypokalémii ve vztahu k LQTS (kongenitálnímu i získanému) jsou považovány již hodnoty pod 4 mmol/l. Podobně se projevuje i **hypomagnezémie**.

6) **Onemocnění srdce**, především srdeční selhávání a nízká ejekční frakce, rovněž i hypertrofie myokardu, komorové arytmie a samozřejmě kongenitální LQTS (jehož výskyt ale není vysoký – cca 1:10 000).

Zásady léčby polékově vyvolaných arytmií

Jak již bylo řečeno výše, výskyt polékové TdP je naštěstí nízký a k jejímu vzniku je nutná kombinace více rizikových faktorů.

Tabulka 2. Faktory zvyšující riziko prodloužení QT intervalu a maligních arytmií

Hraniční nebo dokonce prodloužený QT interval před nasazením léku (QT_c ≥ 0,45 s)
Ženské pohlaví
Vyšší věk
Lékové interakce (blokátory iontových kanálů, inhibitory cytochromu P450)
Hypokalémie, hypomagnezémie
Onemocnění srdce (nízká ejekční frakce, hypertrofie, kongenitální LQTS)

LQTS syndrom dlouhého QT intervalu

Základním pravidlem léčby je odstranění odstranitelných rizikových faktorů. Tzn. vysazení léku s proarytmickým účinkem, substituce kalia až ke hladinám kolem 5 mmol/l (většinou je ale u pacientů s polékovými arytmiemi přítomna hypokalémie pod 3,5 mmol/l). K překlenutí arytmické bouře je vhodné zavedení dočasné kardiostimulace a vnucená stimulační frekvence nad 90/min, která často vede ke stabilizaci rytmu. Přínosná může být i mírná sedace pacienta. Léčba by měla probíhat pokud možno na kardiologickém pracovišti.

Možnosti prevence

Každá smrt vzniklá v souvislosti s podáním léčebného preparátu je velmi závažná. Teoreticky nejbezpečnějším řešením je stažení suspektního preparátu z trhu. Pokud však vezmeme v úvahu výskyt opravdu fatálních případů na jedné straně a úspěšnost léčby daným lékem (např. cisaprid vedl od svého zavedení k výraznému snížení chirurgické léčby reflux ní choroby; ještě v roce 1955 v USA 2/3 pacientů schizofreniků trávilo značnou část svého života v psychiatrických léčebnách, zatímco koncem 80-tých let z nich vyžadovalo ústavní léčbu sotva 5 %)[16], je racionalita takového řešení jistě sporná, zvláště není-li k dispozici alternativní preparát. Navíc vztah léčivo-vedlejší účinek není vždy jednoznačný.

Polékovému prodlužování QT intervalu je v poslední době věnována velká pozornost na všech úrovních. Postoj k tomuto problému zaujala již i Evropská agentura pro hodnocení zdravotnických výrobků (European Agency for the Evaluation of Medicinal Products)[17]. Na kardiologických a především arytmologických sjezdech probíhají sekce věnované tomuto tématu. Odborné společnosti vydávají nejrůznější doporučení týkající se monitorace EKG a testování každé nové chemické entity, která by se mohla stát lékem, stran jejího účinku na repolarizaci kardiomyocytů [18, 19]. I přes pečlivé testování však nebude nikdy zcela vyloučeno riziko, že se proarytmický účinek projeví teprve tehdy, až začne být lék masově používán.

Jako účinná prevence se tedy zatím jeví především opatrný přístup a riziková stratifikace při nasazování preparátů s možným proarytmickým účinkem.

1) V první řadě je nezbytné podrobně prostudovat informace o léčivu poskytované výrobcem. Tento jednoduchý krok není bohužel samozřejmostí.

2) Podrobnou a cílenou anamnézou identifikovat rizikové jedince – viz **Tabulku 2**.

3) Farmakologickou anamnézou zhodnotit riziko interakcí, především se zaměřit na vyloučení kombinace s jiným preparátem prodlužujícím QT interval nebo ovlivňující rychlost metabolizace preparátu. V drtivé většině případů totiž k provokaci arytmií dochází až při kombinaci více rizikových faktorů.

4) Posoudit možnost výskytu elektrolytových dysbalancí – nezapomínat na vliv diuretik, která jsou široce využívána v léčbě hypertenze a srdečního selhání.

5) Stále více expertů doporučuje zavedení rutinního monitorování EKG u rizikových skupin pacientů, kteří mají být léčeni rizikovými léky – především psychofarmaky. Jedinci s QT intervalem delším než 0,45 s již před léčbou jsou považováni za rizikové, je doporučeno u nich provádět kontroly EKG a hladin elektrolytů[14, 20].

6) I v případě, že kritéria 1–5 jsou negativní, je třeba se léčených pacientů při následných kontrolách tázat na případný výskyt varovných příznaků, tedy především synkopy a presynkopy. Proarytmické účinky se totiž mohou projevit až s odstupem a i u pacientů s kongenitálním LQTS s prokázanou mutací může být intermitentně přítomen zcela normální QT interval[21].

Závěr

Fenoménu prodlužování QT intervalu a zvýšení rizika maligních komorových arytmií při léčbě nekardiologickými preparáty je nutné věnovat náležitou pozornost, neboť znamená možné ohrožení života v dobré víře, že léčba bude pro pacienta přínosem. V lékařské populaci je třeba zvyšovat informovanost o tomto jevu, jeho rizikových faktorech a možnostech prevence. Avšak je zároveň nutné vyvarovat se paušálního a úplného odmítnutí rizikových preparátů a vždy pečlivě vážit poměr mezi rizikem a přínosem. U rizikových skupin pacientů léčených rizikovými skupinami léků je třeba uplatňovat velmi individuální přístup.

Ke konečnému zhodnocení celé problematiky bude zapotřebí pokračovat ve výzkumu na všech úrovních, tedy od buněčné membrány až po populaci.

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Jedná se o jednu z největších publikovaných prospektivních studií hodnotících interval QT při léčbě psychofarmaky v běžné klinické praxi.

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Monitoring of QT interval in patients treated with psychotropic drugs

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Abstract

Background: Psychotropic drugs have the potential for QT interval prolongation, the frequency is not known. The aim of this study was to monitor the occurrence of QT interval prolongation in a non-selected population of patients treated with psychotropic drugs with proarrhythmic potential.

Methods: In consecutive patients hospitalized at psychotic wards at the Department of Psychiatry treated with antipsychotic and antidepressant drugs with known or unexplored proarrhythmic potential a 12-lead ECG was recorded (50 mm/s, 20 mm/mV) on therapy; the QT interval was measured manually, corrected according to Bazett and Fridericia. QTc intervals of 470 ms (females) and 450 ms (males) were considered borderline, longer QTc intervals were considered pathologic.

Results: ECGs were recorded in 452 patients (187 females, 265 males, aged 43±16 years). Using Bazett's correction, abnormal QTc values were observed only in 2% of the whole group and in 1.8% of the patients treated with drugs associated with QT prolongation (the greatest QTc value is 490 ms in female and 480 ms in male). With Fridericia's correction, there was only 1 case of borderline QTc in the whole group (the greatest QTc value is 450 ms in both sex groups).

Conclusions: Our 2-year real-life experience shows that occurrence of QTc prolongation in present psychiatric patients is low. Values associated with high risk of arrhythmias (QTc>500 ms) were not observed. This might be related to the recent changes of spectrum of antipsychotic therapy used, the general trend to use lower doses, and increasing awareness about the drug-induced long QT syndrome.

Keywords: Drug induced long QT syndrome; Proarrhythmia; Psychotropic drugs; QT interval

1. Introduction

Many drugs possess an undesirable ability to prolong cardiac repolarization that can be objectively measured as prolongation of the QT interval on the surface electrocardiogram (ECG). A prolonged cardiac repolarization creates electrophysiologic conditions that facilitate the development of arrhythmias, most commonly a polymorphic ventricular tachycardia known as torsade des pointes. By degenerating in ventricular fibrillation, torsade des pointes may cause sudden death [1]. The frequency with which a particular drug induces QT prolongation in a clinically relevant way remains largely unknown.

Psychiatric patients are at an increased risk for sudden death that might be partially attributed to drug-induced torsade des pointes [2,3]. Psychotropic drugs have a known potential for QT interval prolongation. The aim of our study was to monitor the occurrence of QT interval prolongation in a non-selected population of patients treated with psychotropic compounds. The secondary aim was to assess the feasibility of routine QT interval monitoring in this patient population.

2. Methods

2.1. Study population

The study population consisted of all adult patients hospitalized consecutively in psychotic wards at the

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Department of Psychiatry of our hospital. They were treated with different antipsychotic and antidepressant drugs both in monotherapy and in combination. No particular drugs were a priori selected for monitoring. Patients with atrial fibrillation, bundle branch block and pacemaker-dependent rhythm were excluded. The study was approved by the local Ethics Committee of the University Hospital Brno and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. An informed consent was obtained from each patient.

2.2. ECG recordings and QT interval assessments

ECGs were recorded on paper with a 50 mm/s paper speed and 20 mm/mV voltage gain. ECG recordings were evaluated by 2 independent experienced cardiologists blinded to the treatment. QT intervals were measured manually with the precision of 10 ms and corrected according to Bazett and Fridericia formulae and rounded to the nearest 10 ms (in consistence with present clinical practice). QTc intervals of 470 ms in females and 450 ms in males were considered borderline, QTc intervals above these values were considered pathologic.

3. Results

3.1. Group of patients and drugs

Between June 2003 and June 2005, ECG recordings were obtained in 452 patients. Clinical characteristics of study subjects are summarized in Table 1.

Patients were treated with 24 different psychotropic drugs, in the case of 14 of these, cases of QT prolongation have previously been reported. Drugs with known potential for QT prolongation were given to 384 patients. In the case of 6 drugs, torsade des pointes has been reported, and these drugs were taken by 70 patients [4–6]. Fifty patients were treated with combined therapy of 2–3 drugs, in 45 of them, at least 1 drug has been associated with QT prolongation. Numbers of patients treated with individual drug are detailed in Table 2.

Table 1
Clinical characteristics of study subjects ($n=452$)

Male	265
Female	187
Age	43±16
Other diagnoses	
Hypertension	10%
Diabetes	5.5%
CAD	3.5%
Hypokalemia	2.5%
Psychiatric diagnoses	
Schizophrenia	49%
Schizoaffective syndrome	12.5%
Bipolar disorder	21.5%
Other	17%

CAD—coronary artery disease.

Table 2
Psychiatric drugs used in the study

Monotherapy		402 patients	
Combined therapy		50 patients	
Generic name	n	Reported in literature	
		QT prolongation	TdP
Amisulprid	55	+	
Citalopram	39	+	+
Clomipramin	2	+	+
Clozapin	17	+	
Escitalopram	18		
Fluphenazin	1		
Fluoxetine	2	+	+
Haloperidol	10	+	+
Chlorpromazine	2	+	+
Chlorprothixen	3		
Levomepromazine	21		
Milnacipram	7		
Mirtazapine	10		
Olanzapine	91	+	
Oxyprothepine	2		
Paroxetine	1	+	
Quetiapine	55	+	
Risperidone	98	+	
Sertraline	15	+	+
Sulpiride	10		
Tianeptine	9		
Venlafaxine	8	+	
Ziprasidone	11	+	
Zotepine	14		

TdP—torsade des pointes.

3.2. Occurrence of abnormal QTc values

Occurrence of abnormal QTc values is summarized in Table 3. The largest QTc value (Bazett) was 490 ms in a female and 480 ms in a male patient. The largest QTc value (Fridericia) was 450 ms in both sex groups. The average RR interval of the outliers was 708±113 ms. The largest uncorrected QT value was 480 ms (at heart rate of

Table 3
Occurrence of abnormal QTc values in subgroups

	Borderline QTc		Pathologic QTc	
	Bazett	Fridericia	Bazett	Fridericia
Whole group ($n=452$)	5 (1.1%)	1 (0.2%)	4 (0.9%)	0
Patients treated with drugs associated with QT prolongation ($n=384$)	4 (1%)	0	3 (0.8%)	0
Patients treated with drugs associated TdP ($n=69$)	1 (1.5%)	0	0	0
Patients treated with drugs not associated with QT prolongation ($n=64$)	1 (1.55%)	1 (1.55%)	1 (1.55%)	0
Patients treated with drug combination, at least 1 of which associated with QT prolongation ($n=45$)	1 (2.2%)	0	1 (2.2%)	0

QTc—corrected QT interval, TdP—torsade des pointes.

53 beats/min) in a male and 460 ms (at heart rate of 46 beats/min) in a female patient.

4. Discussion

Our observation represents a 2-year real-life single-department experience with a routine QT interval monitoring in patients treated with psychotropic drugs. In all individuals hospitalized at our Department of Psychiatry, ECG is being routinely recorded and QT interval was assessed in order to minimize the risk of proarrhythmia. QT interval monitoring seems appropriate for this purpose since to date, there has not been an example of human TdP induced by a drug that cannot cause QT interval prolongation [7]. Nevertheless, the practicality of the monitoring is complicated by inter-individual differences in drug response that are due to polymorphisms in genes encoding drug metabolizing enzymes, drug transporters, drug targets, as well as repolarization physiology [8,9].

Usually, QT prolongation is considered when QTc (Bazett) interval exceeds 440 ms in males and 460 ms in females [10]. The Committee for Proprietary Medicinal Products also proposed that drug-induced QTc intervals exceeding 500 ms are of concern [11]. These cut-off values were used also in our study. However, there is no well-established threshold duration below which QT prolongation might be considered as benign [12]. Moreover, the best correction approach is still subject of controversy. Only recently it has been showed that QT/RR relationship exhibits a substantial inter-subject variability while also showing high intra-subject stability. Therefore no single mathematical formula can be obtained which will describe the QT/RR relationship satisfactorily in all individuals [13]. Nevertheless, while these findings are useful in the clinical pre-approval drug evaluation, there are of little use in clinical practice when only single ECG is available from each individual. Bazett's correction is therefore frequently used, including described cut-off values, despite numerous reports of its inadequacy and despite the understanding that the QTc interval corrected by Bazett is artificially prolonged at heart rates >60 beats/min and shortened at heart rates <60 beats/min [14]. Fridericia's formula might perhaps be accepted as a practically reasonable correction in clinical setting, although it is still not entirely accurate [15]. In our survey the average heart rate of the outliers was approximately 85 bpm, suggesting that corrected QTc values might have been artificially prolonged because of the correction procedures used.

Psychiatric patients studies in our survey were treated with a wide spectrum of different psychotropic drugs. Majority of these has been reported to influence QT interval [4–6]. Approximately 10% of the patients received combination therapy which is considered as a further risk factor for proarrhythmia. Still, the occurrence of abnormal QTc interval values was low (Table 3). In particular, there was no case of substantial QTc interval prolongation above 500 ms.

In principle, our observation is in agreement with reports on the drugs used. Data on isolated QT prolongation associated with psychopharmacologic compounds are available only for some of them and usually, only cases with QTc prolongation >500 ms are described. Thioridazine is most potent in causing QT prolongation (QTc >450 ms in 60% of patients) [16], while with ziprasidone, QTc intervals >500 ms occur in less than 1% of patients [4]. For clomipramine, there are data in children and adolescents showing QTc prolongation of >450 ms in 11% of individuals [17]. In majority of the other psychotropic drugs, only anecdotal reports are available mostly from cases of poisoning [4–6]. There is only one study dealing with a general psychiatric population, in which in 495 patients QTc prolongation >450 ms were observed in 8% [18]. High proportion of these patients was treated with thioridazine which is presently used very seldom. Low occurrence of QT prolongation in our population is therefore likely explained by a different spectrum of psychotropic drugs used in present psychiatric clinical practice.

Our survey suffers from some important limitations. Firstly, since majority of the patients suffered from chronic psychotic conditions, it was clearly unethical to wash them off therapy for the purposes of baseline ECG recording. Therefore, QT interval change could not have been assessed. In this respect, our survey is a good model of other programs of clinical ECG screening. Therefore an association of a particular drug with QTc value cannot be considered.

Secondly, the size of the group of patients who entered our survey is not particularly large. Nevertheless, this is again a reasonable model of a single-centre clinical experience which is appropriate since clinical ECG monitoring is mostly conducted within individual clinical departments.

Thirdly, because of the above described reasons, it was not possible to confirm the relationship between particular observation of prolonged QTc and a particular drug. Finally, with ECGs taken in chronically treated patients, the subjects prone to QT prolongation might already be dead due to torsade des pointes. There are no meaningful data to quantify this possible effect and it would equally apply to other monitoring experiences.

Hence, our 2-year real-life experience shows that the occurrence of QT prolongation in psychiatric patients subject to contemporary treatment is rather low. This could be related to recent changes of spectrum of antipsychotic drugs used, the general trend to use lower doses of antipsychotic medication and increasing awareness about the drug-induced long QT syndrome. Nevertheless because of possible fatalities the clinical importance of acquired long QT syndrome cannot be underestimated. The low incidence of QT interval prolongation suggests low incidence of torsade des pointes induction in present psychiatric populations but may also lead to an inappropriate underestimation of the

danger of proarrhythmia. In spite of the fact that the incidence of torsadegenic toxicity is likely decreasing, any complacency by the clinical community might have drastic consequences. The need for preventive measures has not diminished. One of the most effective preventive measures in ECG screening in risk groups of patients remains the QT interval monitoring which has been proved fully feasible by our experience.

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(publikovaná původní práce - kvantitativní podíl uchazeče 30 % - koncept, koordinace práce zúčastněných pracovišť, hodnocení EKG a identifikace vhodných jedinců, analýza dat, text publikace)

U jedinců s výskytem polékového prodloužení intervalu QT (nebo i polymorfní komorové tachykardie torsade des pointes) byla provedena mutační analýza genů souvisejících s kongenitálním LQTS. Původní očekávání, že výskyt mutací bude podobný jako u kongenitálního LQTS (tedy 60-70%), se nenaplnila. Mechanizmus získané formy LQTS je tedy komplexnější. Tento fakt je významný i pro chápání molekulárně genetických mechanizmů náhlé srdeční smrti v běžné populaci.

Mutační analýza LQT genů u jedinců s polékovým prodloužením QT intervalu

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Souhrn: *Úvod:* U celé řady nekardiovaskulárních léků bylo popsáno riziko prodloužení QT intervalu, a tím i zvýšení rizika maligních arytmií. Mechanizmus jevu zůstává nejasný. Řada z těchto léků jsou účinné blokátory iontových kanálů kardiomyocytů. Prodloužení repolarizace by tedy mohlo být způsobeno dosud latentními mutacemi genů pro tyto kanály, které se odkryjí až v zátěžové situaci. *Soubor a metody:* V rámci vlastního screeningu pacientů léčených preparáty s proarytmickým potenciálem a sporadických případů odeslaných na naše pracoviště z okresních nemocnic byly celkem u 13 osob zachyceny patologické hodnoty korigovaného QT intervalu (nad 0,44 s u mužů, nad 0,46 s u žen). Jedenáct pacientů dalo svůj souhlas k mutační analýze genů KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 a KCNJ2 (genů asociovaných se syndromem dlouhého QT intervalu). *Výsledky:* Kompletní výsledky mutační analýzy jsou k dispozici do dnešního dne u 8 pacientů. U 5 jedinců byly nalezeny odchylky v sekvenci DNA, které jsou v literatuře označovány jako varianty normy (nukleotidové a aminokyselinové polymorfizmy, varianty intronu). U jednoho muže byla v genu KCNQ1 detekována mutace A590T dosud ve světové literatuře nepopsaná. *Závěr:* Mechanizmus polékového prodloužení QT intervalu je komplexní a nelze jej vysvětlit pouhým postižením na úrovni iontových membránových kanálů.

Klíčová slova: gen – iontový kanál – mutace – proarytmie – QT interval

Mutational analysis of LQT genes in individuals with drug induced QT interval prolongation

Summary: *Background:* In a long list of non-cardiovascular drugs a risk of QT interval prolongation and thus an increased risk of malignant arrhythmias has been described. The precise mechanism remains unclear. Many of these drugs are potent blockers of cardiac ion channels. Thus, prolongation of repolarization could be caused by latent ion channel genes mutations which are revealed under stress conditions. *Group of patients and methods:* Patients were recruited in screening of antipsychotic drugs with proarrhythmic potential, another sporadic cases were reffered from regional hospitals. In 13 individuals pathologic values of corrected QT interval (> 0.44 s in males, > 0.46 s in females) were observed. Eleven patients gave their consent to mutational analysis of KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 and KCNJ2 genes (associated with congenital long QT syndrome). *Results:* At present complete results of mutational analysis are available in 8 patients. In 5 individuals changes in DNA sequence were found which are considered normal variants according to the literature (nucleotide and aminoacid polymorphisms, intronic variants). In 1 male a KCNQ1 gene mutation A590T was identified (yet not reported in literature). *Conclusion:* Mechanisms of drug-induced QT interval prolongation is complex and it cannot be explained simply by ion channel disorders.

Key words: gene – ion channel – mutation – proarrhythmia – QT interval

Úvod

Při podávání celé řady nekardiovaskulárních léků bylo popsáno riziko proarytmie – prodloužení QT intervalu, a tím i zvýšení rizika vzniku maligních arytmií, synkop i náhlé smrti [5,9]. QT interval povrchového elektrokardiogramu (EKG) odráží trvání akčního potenciálu komorových myocytů, především trvání repolarizace. Je to proces tvořený křehkou rovnováhou mezi celou řadou iontových proudů dovnitř a ven z buňky. Z tohoto hlediska je důležité, že společnou vlastností prakticky všech preparátů prodlužujících QT interval je blokáda draslíkového kanálu IKr v srdečních myocytech [1]. Zvýšené riziko maligních arytmií mají především ti jedinci, u nichž po nasazení léčiva dojde k prodloužení korigovaného QT intervalu povrchového EKG, zejména při prodloužení nad 0,5 sekundy [2].

Stejné příznaky (tzn. prodloužení QT intervalu, polymorfní komorová tachykardie torsade des pointes – TdP, synkopy, náhlá smrt) charakterizují také onemocnění zvané kongenitální syndrom dlouhého QT interva-

Tab. 1. Charakteristika vyšetřených jedinců a výsledky mutační analýzy.

				mutační analýza genu					
pacient	pohlaví	QTc (s) arytmie	medikace	KCNQ1	KCNH2	SCN5A	KCNE1	KCNE2	KCNJ2
1.	M	0,45	citalopram	non	IVS8/non	non	D85N/non	non	non
2.	M	0,47	sertralin	Y662Y/non	non	non	non	non	non
3.	Ž	0,48	quetiapin	non	non	non	non	non	non
4.	Ž	0,48	quetiapin	Y662Y/Y662Y	IVS13/IVS13IVS8/non I484I/non F513F/non	non	G38S/non	non	non
5.	M	0,45	venlafaxin	**A590T/non**	non	non	non	non	non
6.	Ž	0,49	cisaprid	non	non	non	non	non	non
7.	Ž	0,5 TdP	terfenadin, itrokonazol	non	Y652Y/Y662Y F513F/non I489I/non	non	non	non	non
8.	M	0,61 TdP	sotalol	non	I489I/I489I F513F/F513F IVS13+22G>A/ IVS13+22G>A	non	G38S/non D85N/non	non	non

TdP – torsade des pointes, tučně je uvedena mutace, šedě aminokyselinové polymorfizmy. V ostatních případech se jedná o nukleotidové polymorfizmy, které nemění sekvenci aminokyselin, nebo varianty intronu.

lu (LQTS). Dnes toto onemocnění chápeme jako společné fenotypové vyjádření nejméně 9 geneticky odlišných nozologických jednotek, jejichž příčinou jsou v převážné většině případů mutace genů kódujících jednotlivé podjednotky iontových membránových kanálů, které hrají důležitou roli v tvorbě a trvání akčního potenciálu kardiomyocytů. V rodinách s výskytem LQTS se nezřídka vyskytují jedinci – nositelé patologických mutací – jejichž QT interval nedosahuje patologických hodnot [8].

Prodloužení repolarizace při tzv. polékovém syndromu dlouhého QT intervalu by tedy mohlo být způsobeno dosud latentními mutacemi genů pro tyto kanály, které se odkryjí až v zátěžové situaci, kterou může být právě podání léku s rizikem proarytmie. Cílem naší studie bylo ověřit tuto hypotézu.

Soubor a metody

V rámci našeho vlastního screeningu pacientů léčených preparáty s proarytmickým potenciálem [7] a sporadických případů odeslaných na naše pracoviště z regionálních nemocnic jsme celkem u 13 osob zachytili patologické hodnoty korigovaného QT intervalu (nad 0,44 s u mužů, nad 0,46 s u žen). QT interval byl odečítán nezávisle na sobě dvěma vyšetřujícími z 12svodového povrchového EKG s posunem 50 mm/s a voltáží 20 mV/mm a korigován dle Bazettovy formule ($QTc = QT/RR^{0,5}$).

Jedenáct pacientů dalo svůj souhlas s odběrem periferní krve k izolaci DNA a s následnou mutační analýzou genů KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 a KCNJ2 (asociovaných s kongenitálním syndromem dlouhého QT intervalu), která byla prováděna metodami v minulosti již podrobně popsanými (PCR, SSCP, DNA sekvenování).

Výsledky

Kompletní výsledky mutační analýzy jsou k dispozici do dnešního dne u 8 pacientů. Výsledky shrnuje tab. 1. U 5 jedinců byly nalezeny odchylky v sekvenci DNA, které jsou v literatuře označovány jako varianty normy (nukleotidové polymorfizmy, varianty intronu, aminokyselinové polymorfizmy). U jednoho muže – pacienta č. 5 – byla v genu KCNQ1 detekována mutace A590T (genotyp A590T/non), dosud ve světové literatuře nepopsaná.

Diskuse

V naší studii jsme provedli mutační analýzu genů pro iontové kanály v malém souboru jedinců, u kterých bylo zachyceno prodloužení QT intervalu při léčbě preparáty s proarytmogenním potenciálem. Pouze v 1 případě mutační analýza odhalila změnu sekvence DNA, kterou je možno označit za mutaci. U dalších 5 jedinců byly nalezeny změny označované jako běžné polymorfizmy. Tyto termíny (mutace, polymorfizmus) samozřejmě odkazují na četnost výskytu, nikoliv na eventuální funkční uplatnění.

Zajímavý je fakt, že ani u 2 pacientů s významným prodloužením QTc a dokonce výskytem TdP nebyla nalezena žádná mutace v analyzovaných genech. Naopak pacient s jen hraniční hodnotou QTc (0,45 s) nositelem mutace je (tab. 1). Tento nález je v současné době nutno brát jako fakt, pro který zatím nemáme uspokojivé vysvětlení.

Dostupná data uvádějí, že mutace genů asociovaných s LQTS jsou nacházeny u 5–10 % osob s polékovými TdP [6,10,11]. Výskyt těchto mutací u jedinců pouze s izolovaným polékovým prodloužením QT intervalu není znám. Dá se však předpokládat, že zde bude ještě nižší. Tomu odpovídají i výsledky naší studie.

I když všechny léky (kardiovaskulární i nekardiovaskulární) dosud asociované s prodloužením QT intervalu jsou účinné blokátory HERG/KCNH2 kanálu [4], nelze fenomén proarytmie vysvětlit pouhým postižením na úrovni iontových membránových kanálů. Mechanizmus polékového prodloužení QT intervalu je komplexní, obecně lze říci, že podkladem interindividuálních rozdílů v reakci na určité léčivo jsou polymorfizmy genů kódujících 1) enzymy metabolizmu léčiv, 2) proteiny účastnící se transportu léků, 3) cílové struktury pro léky [3].

Závěr

Přes pokroky na úrovni molekulární biologie v posledních deseti letech zůstává přesný mechanizmus polékového prodloužení QT intervalu nejasný.

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2.2. Katecholaminergní polymorfní komorová tachykardie (CPVT)

Andrsova I, Valaskova I, Kubus P, Vit P, Gaillyova R, Kadlecova J, Manouskova L, Novotny T. Clinical characteristics and mutational analysis of the RyR2 gene in seven Czech families with catecholaminergic polymorphic ventricular tachycardia. Pacing Clin Electrophysiol 2012;35(7):798-803.

IF 1,746

(publikovaná původní práce - kvantitativní podíl uchazeče 20 % - koncept, koordinace práce zúčastněných pracovišť, vyšetřování pacientů, analýza dat, kritická revize textu publikace)

Práce v úvodu přináší přehled současných poznatků o CPVT. Následuje první systematický popis rodin s touto diagnózou v České republice. Pacienti jsou podrobně charakterizováni klinicky, byla u nich provedena i mutační analýza nejčastěji postiženého genu *RyR2*, a u 2/3 probandů byly nalezeny mutace tohoto genu.

Clinical Characteristics and Mutational Analysis of the *RyR2* Gene in Seven Czech Families with Catecholaminergic Polymorphic Ventricular Tachycardia

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Background: *Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare hereditary arrhythmia. The onset of clinical symptoms usually occurs during childhood, and is typically related to exercise. The aim of our study was to describe the clinical characteristics of seven Czech families with CPVT and the results of mutational analysis of the RyR2 gene in these families.*

Methods: *The subjects and their relatives were investigated at the participating departments. They underwent basic clinical investigation, and history was focused on possible CPVT symptoms, that is, syncopes during exercise. Bicycle ergometry was performed to obtain electrocardiogram recording during adrenergic stimulation. In all the investigated individuals, blood samples were taken for mutation analysis of the RyR2 gene.*

Results: *To date, seven families have been investigated, comprising 11 adults and 13 children. In seven CPVT patients, the indication for examination was syncope during exercise. Diagnosis was confirmed by bicycle ergometry-induced polymorphic ventricular tachycardia. In one relative, polymorphic ventricular tachycardia was also induced. All eight affected individuals were treated with β-blockers and in two, a cardioverter-defibrillator was implanted due to recurrent syncopi. Coding variants of the RyR2 gene were found in four probands.*

Conclusions: *This is a systematic description of CPVT families in the Czech Republic. Our data support the importance of exercise testing for the diagnosis of CPVT. In addition, RyR2 gene coding variants were found in 50% of affected individuals. (PACE 2012; 35:798–803)*

exercise testing, polymorphic ventricular tachycardia, RyR2

Background

Catecholaminergic polymorphic ventricular tachycardia (CPVT) occurring in the structurally intact heart is an inherited cardiac arrhythmic disorder showing a highly malignant clinical course. CPVT can be inherited in an autosomal dominant[1–5] or recessive[6] manner; in some cases, the exact mode of inheritance could not be entirely assessed.[7] The onset of clinical symptoms usually occurs during childhood, and is typically related to exercise. Sudden cardiac death is the first symptom in almost 30% of patients with CPVT.[8] In affected patients, stress-induced ventricular arrhythmias arise from multiple foci and can lead to typical ventricular tachyarrhythmia (VT), the so-called bidirectional VT. These arrhythmias are not sufficiently rapid to disrupt hemodynamics suggesting that the lethal arrhythmia, when it occurs, is ventricular fibrillation. In about 60% of patients with clinical diagnosis of CPVT, mutation of the ryanodine receptor (*RyR2*) gene is present in cases of autosomally dominantly inherited CPVT. The ryanodine receptor is an intracellular calcium channel in the sarcoplasmic reticulum of cardiomyocytes, with important roles in intracellular calcium metabolism. Patients with autosomally recessive inherited CPVT carry mutations of the calsequestrin 2 (*CASQ2*) gene,

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which encodes a Ca^{2+} buffering protein in the lumen of the sarcoplasmic reticulum.[9]

The aim of our study was to describe the clinical characteristics of seven Czech families with CPVT and the results of mutational analysis of the *RyR2* gene.

Methods

Clinical Examination

Probands were identified among syncope cases investigated at the participating departments. All their first-degree living relatives were then investigated according to their age at either pediatric or adult cardiology departments. Basic cardiological investigation was performed in all individuals. History was focused on possible CPVT symptoms (stress syncopi). All individuals were evaluated by bicycle ergometry (off therapy) to obtain electrocardiograms (ECGs) during adrenergic stimulation. Twelve-lead ECG was recorded in modification by Mason-Likar and electronically stored (CardioSys version 2.5, Marquette-Hellige, Freiburg, Germany). Initial stress was 0.5 W/kg, elevated by 0.5 W/kg every 3 minutes. The aim of the stress test was to obtain the maximum heart rate for each age category and gender. All recorded ECGs were printed with a paper speed of 50 mm/s and voltage of 20 mm/mV. Besides common ECG intervals, the QT intervals corrected for heart rate using Bazett's formula and possible Brugada signs were also assessed.[10]

Mutation Analysis

All the clinically investigated individuals gave their informed consent, and peripheral blood samples were taken. Mutation analysis of the *RyR2* gene was performed in all individuals with clinical diagnosis of CPVT confirmed by ergometry. Genomic DNA samples were isolated from peripheral blood lymphocytes. Exons 2–4, 6–15, 17–20, 37, 39–49, 83, 84, 87–105 were amplified by polymerase chain reaction and analyzed by direct sequencing on an ABI PRISM 3130 (Life Technologies, Carlsbad, CA, USA). For mapping of the deletions and duplication in exons 3, 97, and 105, multiplex ligation-dependent probe amplification analysis was used. The detailed methodology has been described elsewhere.[11–14]

Results

Clinical Results

The investigated group consists of 24 members from seven families, 11 adults and 13 children (Fig. 1). Clinical characteristics are summarized in Table I. In the seven probands, the diagnosis was confirmed by ergometry-induced polymorphic ventricular tachycardias (Figs. 2

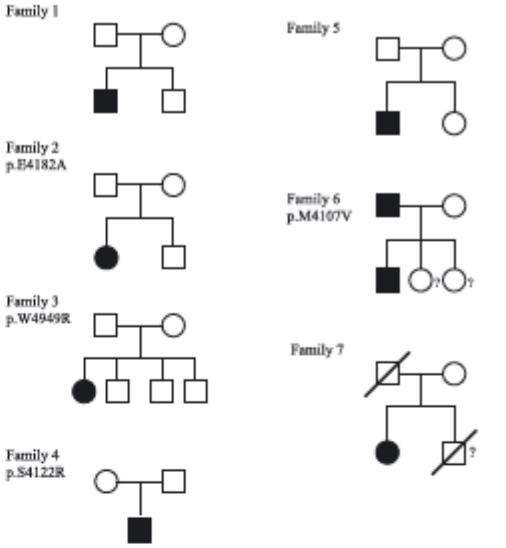

Figure 1. *Pedigrees of families with CPVT diagnosis. Mutations of RyR2 gene are indicated if found. Females are indicated by circles and males by squares. The noninvestigated individuals are denoted by a question mark. Affected individuals are shown as filled symbols and healthy individuals as empty symbols. Deceased individuals are denoted by a slash.*

Table I.

Clinical Characteristics of Investigated Individuals

	Children (n = 13)	Adults (n = 11)
Age	12.82 ± 3.57	42.92 ± 8.50
Age of the first symptoms	7 ± 0.71 (n = 6)	18 (n = 1)
Age of the confirmation of CPVT diagnosis	9.4 ± 2.3 (n = 6)	37.5 ± 0.71 (n = 2)
Syncope	7	1
Native polymorphic VT	7	0
Polymorphic VT induced by ergometry	6	2
Native bidirectional VT	1	0
Bidirectional VT induced by ergometry	4	1
Supraventricular arrhythmias	2	0
β-blockers	7	1
β-blockers and verapamil	1	0
ICD	1	1

VT = ventricular tachycardia; ICD = implantable cardioverter-defibrillator.

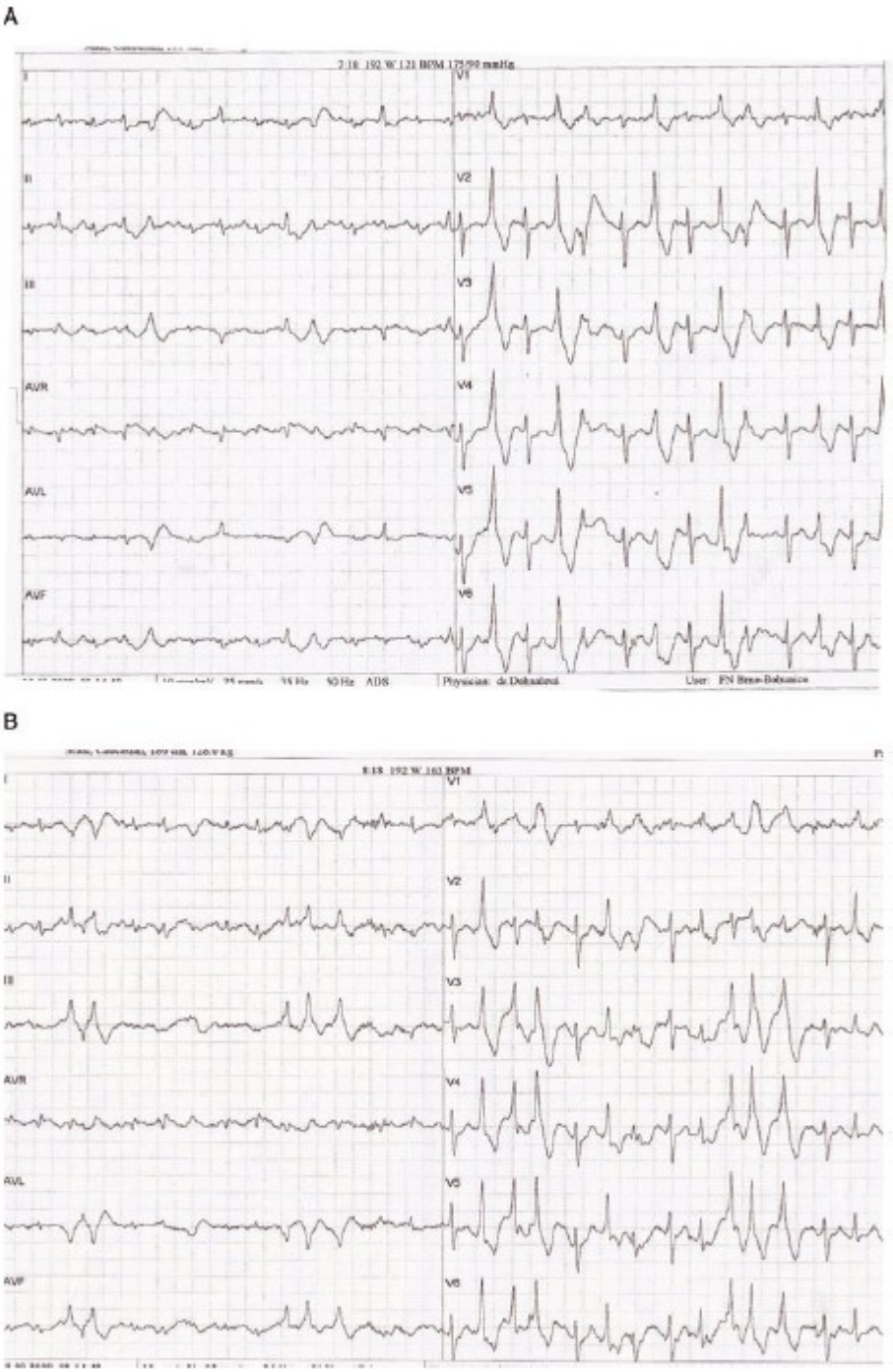

Figure 2. *Examples of exercise-induced polymorphic ventricular arrhythmias in affected individuals. Sweep 25 mm/s, voltage 10 mm/mV.*

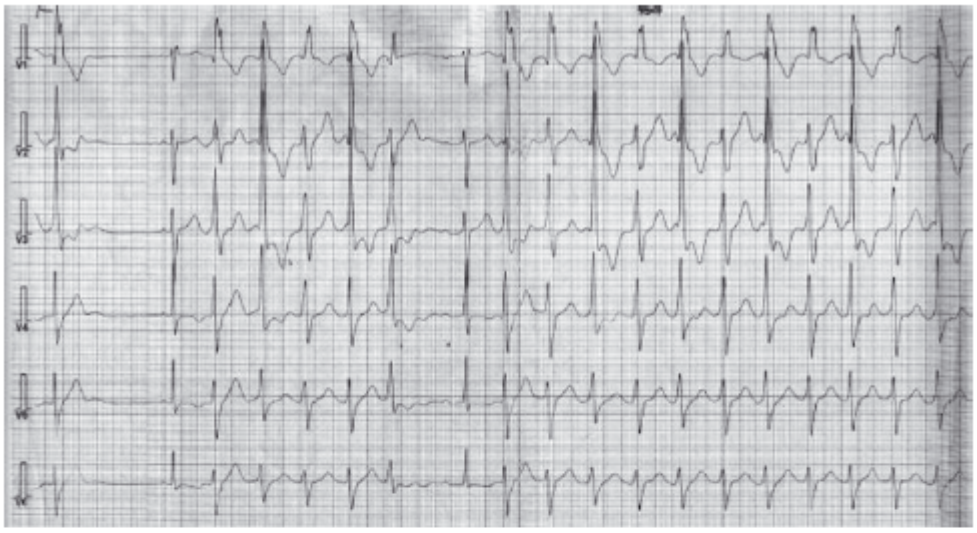

Figure 3. *Exercise-induced bidirectional ventricular arrhythmia in one proband.*

and 3). The test was considered positive if polymorphic ventricular ectopic beats were induced (at least four consecutive beats). In one asymptomatic family member, arrhythmia was also detected during the stress test. None of the investigated individuals exhibited prolonged QTc interval or Brugada ECG and also other ECG features were unremarkable. All eight clinically affected patients were treated with β-blockers: atenolol 25–150 mg per day in four pediatric patients, metoprolol 50 mg per day in other two pediatric patients. In two adult patients, either betaxolol 20 mg per day or metoprolol 200 mg per day were used. In one case, atrial ectopic tachycardia was present and it was effectively suppressed by verapamil. Two patients received implantable cardioverter-defibrillators (ICDs) due to syncope recurrence despite β-blocker therapy.

Mutation Analysis Results

In four of seven probands (57%), coding variants of the *RyR2* gene not previously described were detected (Table II). These four variants were not found in 141 control individuals. The exons containing rare coding variants found in CPVT probands were sequenced in all clinically investigated family members. In none of them, these variants were present. None of the patients with previously implanted ICD was a mutation carrier.

Table II.

List of Mutations of *RyR2* Gene

Nucleotide Change	Amino Acid Change	Exon	Domain
c.[12643A>G]+[=]	p.[M4107V]+[=]	90	III
c.[12545A>C]+[=]	p.[E4182A]+[=]	90	III
c.[12364A>C]+[=]	p.[S4122R]+[=]	90	III
c.[15162T>C]+[=]	p.[W4949R]+[=]	105	IV

Discussion

This study is a systematic description of CPVT families in the Czech Republic. In the majority of cases, exercise-related syncope in childhood was the indication for investigation. In our study, in one patient the CPVT manifested in adulthood, and in another, in the father of a proband who was otherwise asymptomatic, the diagnosis was established only with ergometry. In pediatric patients, diagnosis was established after an average delay of 2 years from the first syncope, since these events were often attributed to vasovagal etiology or to neurological factors.[6] The characteristics of our group are in concordance with data reported previously.[12,13]

Since the resting ECG in CPVT is normal, exercise testing is of great importance. In more than 80% of CPVT patients, complex ventricular

arrhythmias are present during the stress test.[15] This can lead to typical polymorphic ventricular tachycardia-bidirectional ventricular tachycardia. In all our patients, complex forms of ventricular arrhythmias were induced during exercise, emphasizing the role of ergometry in arrhythmologic investigation.

It is important to note that supraventricular arrhythmias and tachycardias are also part of the CPVT phenotype.[2] Indeed, in two of our patients supraventricular arrhythmias occurred.

The molecular pathogenesis of *RyR2*-mediated CPVT underlines the role of increased adrenergic activity as a factor in triggering attacks. In fact, administration of β-adrenergic-blocking drugs is the standard treatment for CPVT, although they are less effective compared to long QT syndrome. In almost 30% of CPVT patients, incomplete protection from exercise-induced arrhythmias or recurrence of stress syncope are observed, and in these cases the implantation of an ICD is indicated.[16] In β-blocker nonresponsive patients, flecainide or left cardiac sympathetic denervation is the treatment of choice.[17] In two of our affected patients, implantation of an ICD was performed, and in the other, treatment with β-blockers was sufficient.

In almost 60% of patients with a clinical diagnosis of CPVT, mutations of the *RyR2* gene are found.[18] The *RyR2* gene is one of the largest genes in the human genome. The majority of mutations appear to cluster in four regions of the predicted RyR2 protein topology, and about 65% of published mutations of the *RyR2* gene are located in these regions.[12,19] Using a tiered targeting strategy suggested by groups in the Mayo Clinic and the Netherlands,[12] we detected different coding variants of the *RyR2* gene in four (57%) of seven probands. Mutations were undetectable in any of the parents, which can be explained either by gonadal mosaicism in one of the parents or by *de novo* mutation. Even the one affected father was not a carrier of his son's mutation. A possibility would be that the proband is a compound or double heterozygote and the father's mutation may be on a nonanalyzed exon.

The most important limitation of our study is a rather small group of investigated individuals. None of the novel mutations identified in our group of Czech CPVT families have been investigated in functional studies and pathogenicity has been suspected based on their absence in control subjects. Tiered targeting strategy can have an impact on the robustness of the estimate of the prevalence of *RyR2* mutations among the cohort. No other CPVT-related genes were analyzed.

In conclusion, in a small group of Czech CPVT patients, the clinical characteristics and percentage of *RyR2* mutations did not differ from internationally published data.

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3. Molekulárně-genetické aspekty komorových arytmií v běžné populaci

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(publikovaná přehledová práce)

Byť byl tento článek publikován již před více než 10 lety, většina tezí v něm uvedených stále platí. Největší změnou je rozpoznání (či spíše tušení) významu tzv. nekódujících sekvencí DNA. Ty tvoří více než 98% lidského genomu a dnes je jasné, že se nejedná o žádný „odpad“ („DNA junk“), nýbrž o velmi důležité regulační sekvence. Většina genů je totiž velmi podobná napříč všemi živočišnými kmeny a geny jsou někdy přirovnávány ke klaviatuře, na kterou lze zahrát Beethovenovu sonátu, ale také jen dětský popěvek. O tom, jaká „skladba“ bude provedena, rozhoduje nejspíše právě informace uložená v oblastech „nekódujících“ sekvencí.

Vnitřní lékařství, 49, 2003, č. 9, s. 768 - 772

Molekulárně genetické aspekty v arytmologii

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Souhrn

Sekvencování lidského genomu bylo dokončeno v roce 2001. Je tedy stanoveno pořadí jednotlivých bází v DNA - neboli známe všechna „písmena" v „knize", ale zatím rozumíme jen omezenému počtu „slov" - neboli byl identifikován jen omezený počet genů. A lidský genom zahrnuje asi 30 000 genů, které navíc díky alternativnímu sestřihu mRNA mohou vést ke vzniku až několika set tisíců různých proteinů. Soubor všech genů daného jedince - genotyp - podmiňuje, jak se pod vlivem prostředí bude utvářet soubor jeho znaků - fenotyp. Co není v genotypu, nemůže být ve fenotypu. V posledním desetiletí došlo k výraznému pokroku v chápaní membránových procesů v souvislosti se studiem monogenně podmíněných arytmických syndromů - především syndromu dlouhého QT intervalu. Ten je způsoben mutací genu pro některý iontový kanál a představuje model arytmogeneze na molekulární úrovni. Komorové arytmie jsou významnou příčinou úmrtí pacientů s kardiovaskulárními chorobami. Nové studie ukázaly, že i v této populaci je výskyt maligních arytmií familiárně závislý a tedy do značné míry geneticky podmíněný. Náhlé úmrtí rodiče zvyšuje 1,8krát riziko náhlého úmrtí pro potomka. Při náhlém úmrtí obou rodičů pak riziko pro potomstvo dosahuje až hodnoty 9,4. Genetická variabilita rizika náhlé smrti se kromě procesů tvorby a propagace elektrického impulsu v myokardu ve může uplatňovat ještě v následujících oblastech: 1. tvorba a stabilita aterosklerotického plátu, trombogeneze a ischemie v koronárním řečišti, 2. řízení excitability myokardu a cévní motoriky. Cílem nynějšího a budoucího výzkumu je nalezení dědičných „molekulárních" ri zik arytmií. Pochopení této úrovně patofyziologických procesů otevře cestu nové generaci diagnostických i terapeutických metod.

Klíčová slova: Arytmie - Gen - Iontový kanál - Náhlá smrt

Summary: Novotný T.: Molecular Genetic Aspects in Arrhythmology

The sequencing of human genome was completed in 2001. The position of particular DNA base is established - i. e. we know all "letters" in the "book" but we understand only limited number of "words" i. e. only limited number of genes was identified. And the human genome consists of about 30 000 genes from which through the mechanism of alternative RNA splicing more than 100 000 genes can be derived. All the genes of one individual form the genotype. The expression of genotype in particular environment forms the phenotype. What is not present in genotype can neither be present in phenotype. In the last decade a substantial progress was achieved in understanding of membrane processes mostly due to research of relatively rare inherited monogenous arrhythmic syndromes - first of all the long QT syndrome. It is caused by mutations in ion channel genes and it provides a model of arrhythmogenesis on molecular level. Ventricular arrhythmias are important cause of mortality in patients with cardiovascular diseases. New studies have provided strong evidence for familial sudden cardiac death (SCD) aggregation and therefore also genetic influence. Parental history of SCD increases the relative risk of SCD for offsrping to 1.8. In the case of both maternal and paternal SCD events the risk for offspring is a remarkable 9.4. There are 3 pathways by which genetic variation may contribute to risk for SCD: 1. alterations in electrogenesis and conduction, 2. formation and stability of atherosclerotic plaque, thrombogenesis and ischemia within the coronary circulation, 3. control of myocardial excitability and vascular motorics. The main objective of both today and future research is identification of inheritable "molecular" risk factors of arrhythmias. Understanding of this level of pathophysiological processes will subsequently lead to new generation of both diagnostic and therapeutic methods.

Key words: Arrhythmias - Gene - Ion channel - Sudden death

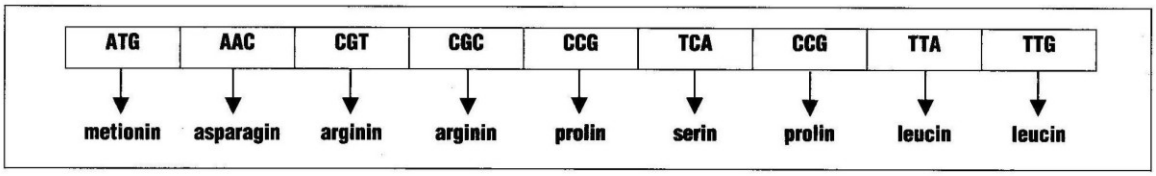
Vnitř. Lék., 49, 2003, No. 9, p. 768 - 772

Úvod

Na rok 2003 připadají dvě významná biologicko-medicínská výročí: před 100 lety Einthoven publikoval první práci o využití elektrokardiografie (EKG) (1) a před 50 lety Watson s Crickem rozluštili genetický kód nukleových kyselin (18).

Arytmologie - jakožto podobor kardiologie - je založena právě na interpretaci EKG záznamu, ať už povrchového nebo intrakardiálního. Každá EKG křivka je výsledkem procesů, které v srdci probíha-

Předneseno na 124. internistickém dnu - XIX. Vanýskově dnu „Elektrické srdeční potenciály - 100 let EKG v interní praxi" dne 21. března 2003 v Brně.



Obr. 1. Genetický kód - trojice bází DNA - tzv. kodon - určuje konkrétní aminokyselinu a její postavení v řetězci bílkoviny

jí na molekulární úrovni - tedy výsledkem toku iontů bílkovinnými kanály napříč buněčnou membránou. Struktura všech bílkovin je zakódována v pořadí nukleových kyselin - v genech - které každý jedinec dědí po svých rodičích (obr. 1).

Přísloví „Jablko nepadá daleko od stromu" vyjadřuje, že lidé si již odedávna uvědomují fenomén dědičnosti. Teprve však před půldruhým stoletím Mendel položil základy nové vědy o dědičnosti - genetiky. Dnes je genetika nedílnou součástí medicíny a odběrem rodinné anamnézy ji praktikuje každý lékař. Významným milníkem v dějinách tohoto oboru je dokončení sekvence lidského genomu (6, 17).

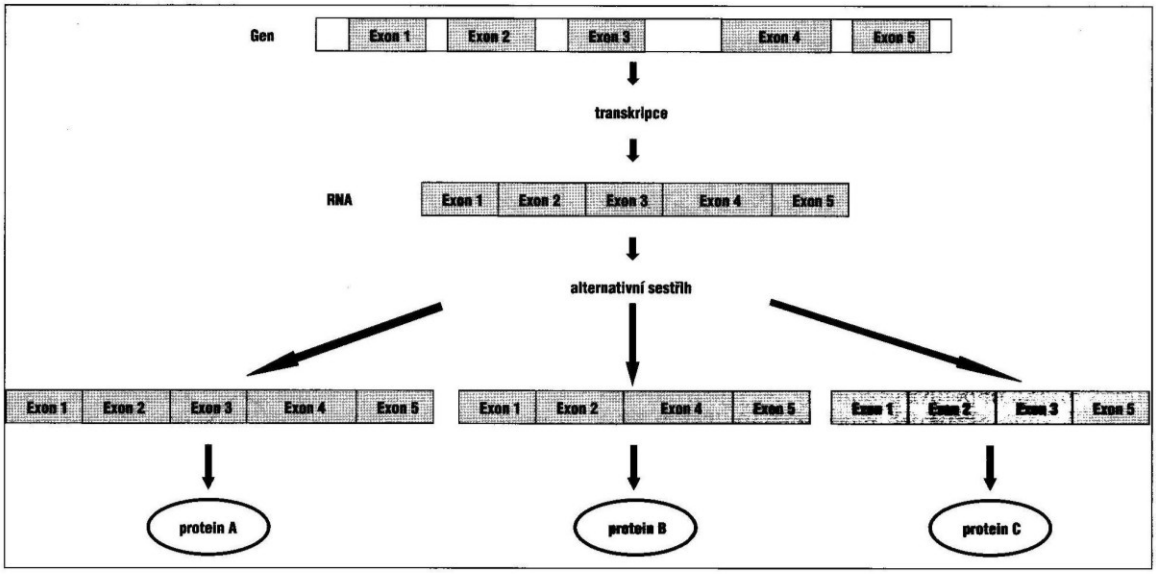
Téma tohoto článku je velmi obsáhlé, není tedy možno se vyhnout často i radikálnímu zestručnění některých problémů.

Lidská genetická informace - lidský genom

Sekvencování lidského genomu bylo dokončeno v roce 2001. Je tedy stanoveno pořadí jednotlivých bazí v deoxaribonukleové kyselině (DNA) - neboli známe všechna „písmena" v „knize", ale zatím rozumíme jen omezenému počtu „slov" - neboli byl identifikován jen omezený počet genů. A lidský genom zahrnuje asi 30 000 genů, které navíc díky alternativnímu sestřihu messengerové ribonukleové kyseliny (mRNA) a posttranslačním úpravám mohou vést ke vzniku až několika set tisíců různých proteinů (obr. 2). Soubor všech genů daného jedince - genotyp - podmiňuje, jak se pod vlivem prostředí bude utvářet soubor jeho znaků - fenotyp. Co není v genotypu, nemůže být ve fenotypu.

Molekulární arytmologie

Pro arytmologii má z molekulárně-genetického pohledu zásadní význam výrazný pokrok v chápaní membránových procesů, k němuž došlo v posledním desetiletí. Byla identifikována řada jednotlivých iontových proudů a jejich proteinových kanálů, které se podílejí na tvorbě akčního potenciálu. U většiny z nich známe alespoň gen kódující jejich hlavní proteinovou podjednotku. Zásadní poznatky v této oblasti byly získány zejména při výzkumu relativně vzácných monogenně podmíněných arytmických syndromů - především syndromu dlouhého QT intervalu (LQTS) a Brugada syndromu. Ty jsou způsobeny mutací genu pro některý iontový kanál a tak představují model pro studium procesů arytmogeneze na molekulární úrovni (10, 11).



Obr. 2. Alternativní sestřih RNA - jeden gen může dát vznik více příbuzných proteinů. Exon - kódující úsek genu

Syndrom dlouhého QT intervalu a Brugada syndrom

Pacienti trpící těmito chorobami mají vysoké riziko maligních komorových arytmií a náhlé srdeční smrti (NSS). Jak bylo uvedeno výše, obě onemocnění řadíme mezi tzv. monogenní poruchy. Znamená to, že jejich příznaky je možno v zásadě vysvětlit postižením jediného konkrétního genu. U LQTS byly doposud identifikovány mutace 5 genů pro kanálové proteiny nesoucích iontové proudy INa, IKs a IKr v srdečních myocytech (tabulka 1). U Brugada syndromu byly prokázáno postižení INa odlišného typu než u LQTS.

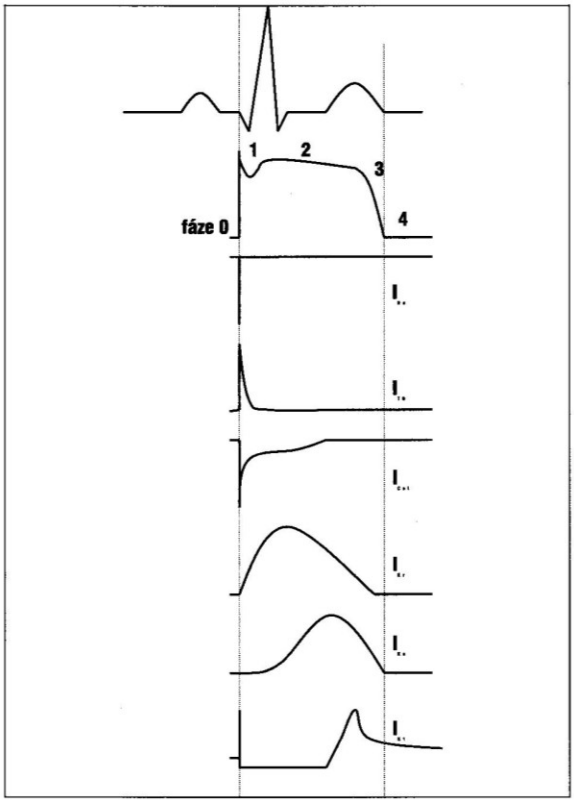
Tab. 1. Typy LQTS ve vztahu ke genům a jejich produktům

Typ LQTS	Chromosom a lokus	Gen	Iont. kanál
Jervell a Lange-Nielsen syndrom			
JLN 1	11p15.5	KCNQ1 (KVLQT1)	α I_{Ks}
JLN 2	21q22.1-22.2	KCNE1	β I_{Ks}
Romano a Ward syndrom			
LQT 1	11p15.5	KCNQ1 (KVLQT1)	α I_{Ks}
LQT 2	7q35-36	KCNH2 (HERG)	α I_{Kr}
LQT 3	3p21-24	SCN5A	α I_{Na}
LQT 4	4q25-27	?	?
LQT 5	21q22.1-22.2	KCNE1	minK (β I_{Ks})
LQT 6	21q22.1	KCNE2	MIRP (β I_{Kr})
LQT 7	???		

Jedinci s Jervell – Lange-Nielsen syndromem jsou homozygoti, Romano Ward pak heterozygoti.
α, β – podjednotky iontových kanálů, I_{Ks} – pomalu se aktivující opožděný rektifikující K^+ proud fáze 3 akčního potenciálu kardiomyocytů, I_{Kr} – rychle se aktivující rektifikujíc K^+ proud fáze 3 akčního potenciálu kardiomyocytů, I_{Na} – rychlý Na^+ proud fáze 0.

Klidový i akční potenciál (AP) srdečních myocytů je tvořen křehkou rovnováhou mezi iontovými proudy dovnitř a ven z buňky (obr. 3). Poškození kteréhokoli z nich může tuto rovnováhu narušit a vést tak ke změně trvání AP. Prodloužení AP a tím i prodloužení QT intervalu tedy teoreticky může být způsobeno jak zesílením nebo prodloužením depolarizačních proudů (tedy INa a ICaL), tak oslabením či zkrácením repolarizačních proudů (především ITO1, IKr a IKs).

Nejdůležitějšími ionty v tvorbě klidového i akčního potenciálu (AP) jsou kationty sodný (Na^+), draselný (K^+) a vápenatý (Ca^{2+}), které jsou po obou stranách membrány rozloženy nerovnoměrně, čímž vznikají napěťové gradienty. Na jejich udržení se podílí řada aktivních mechanismů. Tak v okamžiku klidového potenciálu je vně buněčné membrány výrazná převaha Na^+ a Ca^{2+}, uvnitř pak převaha K^+. Pro transport iontů přes buněčnou membránu jsou důležité specializované bílkovinné struktury - iontové kanály. Po otevření těchto kanálů tečou příslušné iontové proudy ve směru gradientů napříč membránou. Pro elektrofyziologii AP jsou důležité především tzv. „napěťově vrátkované (voltage gated)" iontové kanály. To znamená, že se otevírají a zavírají v závislosti na aktuálním napětí na buněčné membráně, tedy vlastně v závislosti na přesunech iontů přes membránu. Tyto kanály jsou navíc vysoce selektivní pro konkrétní iont. Tradičně se na AP popisuje 5 fází: fáze 0 - rychlá depolarizace, fáze 1 - časná repolarizace, fáze 2 - „plateau", fáze 3 - pozdní rychlá repolarizace, fáze 4 - klidový membránový potenciál. Klidový membránový potenciál (méně než -80 mV) je vytvářen především činností Na^+/K^+ pumpy, dále pak jsou v této fázi otevřeny některé K^+ kanály. V okamžiku, kdy membránový potenciál činností pacemakerových center dosáhne -70 mV, otevírá se Na^+ kanál. Výsledkem je mohutný ale velmi krátce trvající (cca 2 ms) proud sodíku do buňky (INa) vedoucí k depolarizaci buněčné membrány. Vzápětí se s různým zpožděním otevírají repolarizační, převážně K^+ kanály. Jako první je to tzv. „přechodný proud ven z buňky" - transient outward (ITO1 a ITO2), výsledkem je fáze 2 - rychlý, ale krátce trvající pokles AP. Zároveň se totiž začíná otevírat L typ Ca^{2+} kanálu (ICaL), který po nějakou dobu brání dokončení repolarizace (fáze plateau). Mezitím nabývá na síle tzv. opožděný rektifikující (delayed rectifier - DR) K^+ proud (IK), který má v komorových myocytech 2 složky - rychle (rapidly) se aktivující DR (IKr) a pomalu (slowly) se aktivující DR (IKs). Především ty pak dokončují návrat membránového potenciálu ke klidovým hodnotám (12)



Obr. 3. Klidový a akční potenciál kardiomyocytů

Prodloužení trvání repolarizace, tedy prodloužení QT intervalu, usnadňuje vznik arytmií na podkladě tzv. časných následných depolarizací - „early afterdepolarizations" - kdy dochází ke znovuotevření depolarizujících kanálů (INa nebo ICa) ještě před ukončením repolarizace, tedy před „dokončením" AP. Vzniká tak nepravidelný sled rychle po sobě se opakujících depolarizací, které mohou vést ke vzniku polymorfní komorové tachykardie typu „torsade de pointes" (TdP). K tomuto předčasnému otevření výše uvedených kanálů dochází při takovém prodloužení repolarizace, které časově přesáhne dobu nutnou ke zotavení depolarizačních kanálů a tak umožní jejich opětné otevření.

Postižení dosud známých genů vysvětluje klinický obraz LQTS jen u asi 50 % pacientů a dokonce jen u 30 % pacientů s Brugada syndromem. U ostatních předpokládáme podobný mechanismus, konkrétní geny však dosud neznáme. Velice pravděpodobně lze uvažovat i o kombinaci postižení více genů nejen pro iontové kanály, ale i např. jejich regulační proteiny. I u dosud známých typů LQTS totiž klinické vyjádření u různých členů téže rodiny výrazně kolísá, i když jsou nositeli téže mutace. Např. ve 24členné rodině sledované na našem pracovišti je 9 nositelů mutace genu KCNQ1, z nichž u jedné ženy si recidivující arytmie vyžádaly implantaci kardioverteru-defibrilátoru, u jiné nositelky stejné mutace naopak dosud nebylo zachyceno ani prodloužení QT intervalu (16). Je tedy zřejmé, že i obraz choroby, kterou považujeme za monogenní, je ve skutečnosti výsledkem kombinace mnoha příčin. Z genetického hlediska hovoříme v těchto souvislostech o tzv. genovém pozadí. Tento poněkud vágní termín vyjadřuje naše nedostatečné znalosti a pochopení problému.

LQTS a Brugada syndrom jsou také někdy označovány jako elektrická onemocnění srdce, neboť komplexním kardiologickým vyšetřením u těchto pacientů neprokážeme strukturální onemocnění srdce.

Náhlá srdeční smrt na molekulární úrovni u strukturálních onemocnění srdce

Ačkoli k našemu chápání arytmogeneze přispělo především studium relativně vzácných elektrických onemocnění srdce, jsou maligní komorové arytmie především významným mechanismem úmrtí pacientů, kteří trpí společensky významnými akutními i chronickými onemocněními srdce. Jsou zodpovědné minimálně za polovinu úmrtí pacientů s ischemickou chorobou srdeční a kardiomyopatiemi (19). Nedávno byly publikovány výsledky 2 geneticky zaměřených populačních studií (2, 4). Multivariační analýza konvenčních rizikových faktorů pro ICHS (faktory biologické, dietetické a faktory prostředí) ukázala, že pozitivní rodinná anamnéza náhlé srdeční smrti (NSS) je silným nezávislým rizikovým faktorem NSS pro potomstvo. Anamnéza náhlého úmrtí u rodiče zvyšuje 1,8krát riziko náhlého úmrtí pro potomka. V případě náhlého úmrtí obou rodičů pak relativní riziko pro potomstvo dosahuje překvapivě vysoké hodnoty 9,4. Přes problémy s označením konkrétního úmrtí za „náhlé" bezpochyby tyto studie podávají přesvědčivý důkaz o familiárním výskytu NSS i v běžné populaci (14). Při vědomí familiárně společně sdílených vnějších rizikových faktorů je tedy zřejmá existence geneticky podmíněných odchylek fyziologických procesů zvyšujících riziko NSS. Mutace jednotlivých genů zde pravděpodobně hrají jen malou roli. Spíše se jedná o výsledek společného působení mnoha genů, o polygenní etiologii. V tomto případě se pak mohou uplatňovat i tzv. běžné polymorfismy DNA (tj. odchylky vyskytující se v různém procentu v populaci a považované za varianty normy, někdy hovoříme též o tzv. intermediárních fenotypech).

Genetická variabilita rizika náhlé smrti se kromě výše zmíněných 1. procesů tvorby a propagace elektrického impulsu v myokardu ve může uplatňovat ještě v následujících oblastech: 2. procesy a faktory tvorby a stability aterosklerotického plátu, trombogeneze a ischemie v koronárním řečišti, 3. centrální i místní řízení excitability myokardu a cévní motoriky.

Ad 1) Jedinci s vyšším rizikem NSS teoreticky mohou být nositeli dosud latentních mutací v genech pro iontové kanály, např. právě těch, o kterých už víme, že se uplatňují v patogenezi LQTS a Brugada syndromu. Dále to mohou být i regulační proteiny iontových kanálů nebo proteiny cytoskeletu, které kanály zakotvují v jejich pozicích (8).

Ad 2) Ateroskleróza, trombóza a infarkt představují populačně nejvýznamnější příčinné faktory letálních arytmií. Výzkumu těchto procesů na molekulární úrovni je věnována velká pozornost a seznam kandidátních genů, jejichž mutace nebo i polymorfismy mohou měnit maligním způsobem průběh choroby, je velmi dlouhý. Přímá souvislost těchto patologií s NSS zatím studována nebyla, nicméně jsou známy asociace s vyšší kardiovaskulární mortalitou např. u polymorfismů lipoproteinů a jejich receptorů (15). Užší vazbu na riziko NSS budou mít jistě faktory přímo ovlivňující riziko ruptury aterosklerotického plátu. Jsou např. známy dědičné odchylky metaloproteináz intercelulární matrix, které podporují odbourávání vazivové čepičky plátu (3). Řadu podobných příkladů je možno uvést pro procesy trombogeneze.

Ad 3) Příkladem variability autonomního nervového systému je polymorfismus (Ile164) β2-adrenergního receptoru, který je u pacientů se srdečním selháním asociován se zvýšenou mortalitou (7). Intenzivně studovaným působkem je také endothelin. Výskyt jedné z jeho variant je vyšší u jedinců se špatnou tolerancí komorových tachykardií (5).

Je třeba mít stále na paměti, že ač známe asociace některých mutací či polymorfismů s určitým klinickým stavem, neznáme většinou vlastní

mechanismus, jakým se tyto mutace či polymorfismy uplatňují.

Fenomén proarytmie

Důležitou roli hraje i výzkum fenoménu proarytmického působení nejrůznějších, nejen antiarytmických, preparátů. Jedná se o rozsáhlé téma zasluhující samostatný článek. Zájemce o podrobnější informace odkazuji na článek věnovaný problematice proarytmií v tomto čísle Vnitřního lékařství (13) a na naši práci publikovanou v roce 2002 v časopise Česká a slovenská psychiatrie (9).

Závěr

Stojíme na počátku nové éry. Cílem nynějšího u budoucího výzkumu je identifikace dědičných „molekulárních" rizik maligních arytmií a odkrytí mechanismů, kterými se jednotlivé polymorfismy uplatňují v určování úrovně intermediárních fenotypů a tím i v určení míry rizika. Pochopení této úrovně patofyziologických procesů otevře cestu nové generaci diagnostických i terapeutických metod.

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3.1. Výskyt variant genů pro iontové kanály u pacientů s vysokým rizikem náhlé smrti

Jak bylo uvedeno ve výše vložené přehledové práci, existují dnes přesvědčivé důkazy o familiárním výskytu náhlé srdeční smrti v běžné populaci. V roce 1998 navrhl Roden koncept repolarizační rezervy (23): ve zdravém srdci zajišťují normální průběh repolarizace vícečetné redundantní mechanizmy. Porucha jednoho z těchto mechanizmů nevede k poruše repolarizace ani ke změně intervalu QT. Avšak v zátěžových podmínkách (např. ischémie či hypokalémie) mohou být kompenzační mechanizmy vyčerpány a dosud latentní mutace genů pro iontové kanály může zvýšit riziko maligních arytmií.

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IF 1,351

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Práce hodnotí výskyt kódujících variant v genech pro iontové kanály myokardu u selektovaných pacientů se strukturálním onemocněním srdce a nízkou ejekční frakcí, kteří byli úspěšně resuscitováni pro dokumentovanou fibrilaci komor, tedy přežili náhlou smrt. Kódující varianty genů asociovaných s LQTS byly u nich nalezeny signifikantně častěji než v kontrolních populacích, což potvrzuje mechanistickou roli těchto genů v patofyziologii náhlé smrti u pacientů s ischemickou chorobou srdeční.

Mutation Analysis Ion Channel Genes Ventricular Fibrillation Survivors with Coronary Artery Disease

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Background: *Observations from population-based studies demonstrated a strong genetic component of sudden cardiac death. The aim of this study was to test the hypothesis that ion channel genes mutations are more common in ventricular fibrillation (VF) survivors with coronary artery disease (CAD) compared to controls.*

Methods: *The entire coding sequence of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 genes was analyzed in 45 (five females) CAD individuals—survivors of documented VF and in 90 matched healthy controls. In another control group of 141 matched patients with CAD without malignant arrhythmias, the exons containing rare coding variants found in the VF survivors were sequenced.*

Results: *The carrier frequency of all the rare sequence variants was significantly higher in the VF survivors (8/45, 17.8%) than in CAD controls (3/141, 2.2%, $P = 0.001$). In VF survivors, four coding variants in eight individuals were found. Three in KCNH2 gene: R148W and GAG186del are novel; P347S was previously related to long QT syndrome. In SCN5A gene, P2006A variant was found in five unrelated males. This variant has been demonstrated previously to have small effect on sodium channel kinetics. No rare coding variants were found in the healthy controls. The P2006A variant was found in three CAD controls.*

Conclusion: *The prevalence of selected, rare coding variants in five long QT genes was significantly higher in cases versus controls, confirming a mechanistic role for these genes among a subgroup of patients with coronary disease and VF. (PACE 2011; 34:742–749)*

sudden death, ventricular fibrillation, ion channel, mutation

Introduction

Approximately 50% of all coronary heart disease deaths are sudden. Each year, several hundred thousand individuals die due to sudden cardiac death (SCD) in both the United States and the European Union.[1] Observations from population-based studies demonstrate a strong genetic component of SCD.[2,3,4,5] It is clear that there must be genetically determined variations of physiological processess that increase the risk of SCD. One of the pathways by which genetic variation in pathophysiological mechanisms may contribute to increased risk of SCD is electrogenesis and conduction in myocardium. It is well known that myocardial ion channel gene mutations lead to increased SCD risk in patients with relatively rare diseases, such as long QT syndrome (LQTS) and Brugada syndrome. In some LQTS families, up to 75% of carriers of pathologic mutations remain completely asymptomatic, that is, they did not even present with prolonged QT interval.[6] Therefore, a normal QT interval value does not automatically exclude that particular individual as a carrier of potentially dangerous mutation. Recently, it has been shown that the prevalence of LQTS is at least 1:2500. Similarly, in the general population the prevalence of ion-channel gene polymorphisms may be much higher than previously thought.[7] Such variants could predispose individuals in the general population

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to SCD. In the present study, we performed mutational analysis of five LQTS-related myocardial ion-channel genes in patients with coronary artery disease (CAD) and ventricular fibrillation (VF) documented at the time of SCD event and in a control population.

Methods

Investigated Subjects

The group of patients consisted of 45 Caucasian patients (five females) from the region of south Moravia in the Czech Republic (population approximately 1.5 million individuals). The primary inclusion criterion was VF documented at the time of circulatory arrest not related to acute phase of myocardial infarction (i.e., more than 48 hours after index event). Such individuals were identified from the implantable cardioverter defibrillator (ICD) registry of our department, partially retrospectively (years 1998–2006); from 2007 new consecutive patients were included prospectively. Two patients suffered VF while staying in a hospital for more than 72 hours after acute myocardial infarction. All other individuals suffered circulatory arrest in the out-of-hospital setting; VF was recorded by emergency car staff. Patients were successfully resuscitated and then transferred to our department, either directly or via regional hospitals. After circulatory stabilization and improvement in mental status, all patients underwent comprehensive cardiological examination. None of the patients had an acute myocardial infarction at the time of arrhythmia according to electrocardiogram (ECG) and serum troponin level analysis. In all patients, CAD was confirmed by coronary angiography findings of significant stenoses (>70%) or chronic occlusions. The left ventricle ejection fraction (LVEF) was calculated from echocardiography analysis. In three patients, QT interval assessment was not possible due to left bundle branch block or atrial fibrillation. None of the other 42 patients showed substantial QT interval prolongation or Brugada-like ECG.

A total of 231 geographically, sex-, and age-matched control individuals were divided into two groups. The first control group consisted of 90 individuals who underwent basic medical investigation and were assessed as healthy; detailed ECG information was not available for this cohort. The second group consisted of 141 patients with CAD who were hospitalized at our department owing to an acute coronary event. For all patients, the diagnosis was confirmed by coronary angiography. All patients had depressed LVEF and they were alive at least 3 years after the index event. Thus, they could be considered as a CAD group with a low risk of SCD. Clinical characteristics of both groups are summarized in Table I

Table I.

Clinical Characteristics of Investigated Individuals

	SCD Survivors (n = 45)	Healthy Controls (n = 90)	CAD Controls (n = 141)
Male/female	40/5	80/10	129/12
Age	63.2 ± 7.9	66.6 ± 6.2	66.7 ± 10.7
Left ventricle ejection fraction	40 ± 12%	–	42 ± 9%
History of myocardial infarction	32 (71%)	0	127 (90%)

SCD = sudden cardiac death; CAD = coronary artery disease.

Informed consent was obtained from all the individuals and peripheral blood samples were taken for genomic DNA preparation. The study protocol was approved by the Ethical Committee of the University Hospital Brno and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Genomic DNA Samples and Polymerase Chain Reaction Amplification

The coding sequences of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* genes were analyzed (GenBank accession nos. AF000571, NM000238, NT022517, NM000219, NM005136). Genomic DNA was extracted from peripheral blood samples according to a standard protocol using DNA BloodSpin Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), and the classical method of ethanol progression. Exons of *KCNQ1*, *KCNH2*, and *KCNE1* genes were amplified by multiplex polymerase chain reaction (PCR); previously reported intronic oligonucleotide primers were used.[8,9] A total of 34 intronic oligonucleotide primer pairs were used to amplify the coding area of *SCN5A* (modified according to reference 10) The PCR amplifications were performed using *Taq* DNA polymerase (Fermentas, Inc., Glen Burnie, MD, USA) and HotStarTaq Master Mix (Qiagen, Inc., Valencia, CA, USA). Each PCR was performed in 200-μL thin-walled PCR tubes in a total reaction volume of 25 μL. Each PCR amplification was performed using a SensoQuest Labcycler (Progen Scientific, Ltd., Mexborough, UK) and a Perkin Elmer 2400 machine (Applied Biosystem, Foster City, CA, USA).

Mutation Analysis

Single-strand conformation polymorphism (SSCP) and DNA sequence analyses were used to screen for *KCNQ1, KCNH2, SCN5A,* and *KCNE1* gene mutations. In the case of exons 15, 17-1, and 23 of *SCN5A* and exon 3 of *KCNE1*, the high-resolution melting (HRM) method was used.

SSCP Analysis

For the analysis of all genes, 2-μL aliquots of the amplified samples were mixed with 4 μL of bromphenol blue loading dye (95% formamide; 10 mmol/L ethylenediaminetetraacetic acid (EDTA); 0.1% bromphenol blue; 0.1% xylene cyanol) and were denatured at 94°C for 5 minutes, then cooled rapidly on ice and held for 5 minutes. Denatured multiplex PCR samples of *KCNQ1* were analyzed in multiplex SSCP in 10% and 12% polyacrylamide gels (crossling 40:1) at 10°C and 15°C/12 h/120 V. The fragments shown to have single-strand mobility in multiplex SSCP were reanalyzed by separate exon SSCP analysis under the same electrophoretic conditions. Analysis of multiplex PCR of *KCNH2* gene was performed using 8% polyacrylamide gel (crossling 50:1) at 8.5°C/3 h/40 W followed by separate exon SSCP analysis under the same electrophoretic conditions.

Denatured samples of the *SCN5A* gene were loaded into nondenaturing polyacrylamide gels with two layers (5% and 9%) and run at 210 V and 10°C for 6–8 hours according to the length of each fragment (Multigel-Long, Whatman Biometra, GmbH, Göttingen, Germany).

The bands were visualized by silver staining.

HRM Analysis

HRM analysis was performed using a LightCycler 480 real-time PCR system and high-resolution Melting Master Kit (Roche Diagnostics, GmbH, Mannheim, Germany). PCR products were used as loaded samples. Primers were the same as those used for SSCP.

DNA Sequencing

In cases of different band patterns, sequencing was performed with forward and reverse primers. To reduce the risk of polymerase-induced errors and false-positive results, the PCR products suspected of sequence change were reamplified before sequencing.

For purification of the amplified samples, MinElute PCR Purification Kit (Qiagen, Inc.) was used. Cycle sequencing was performed using the Big Dye Terminator Kit (Applied Biosystems), and the DyeEx2.0 Spin Kit (Qiagen, Inc.) was used for purification of samples after cycle sequencing. Exons were sequenced using the ABI PRISM 310 instrument (Applied Biosystems).

The resulting sequences of the screened *KCNQ1, KCNH2, SCN5A,* and *KCNE1* genes were aligned with the wild-type sequences deposited in NCBI (GenBank accession nos AF000571, NM000238, NT022517, NM000219).

Statistics

The distributions of allelic frequencies and their differences were calculated using χ^2 tests. Odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the risks associated with the detected polymorphisms. To calculate the significance of OR, Fisher's exact test was used. The program package Statistica v. 8.0 (Statsoft Inc., Tulsa, OK, USA) was used for all statistical analyses.

Results

Coding Variants in VF Survivors

The full coding sequences of *KCNQ1, KCNH2, SCN5A, KCNE1,* and *KCNE2* genes of all the VF survivors were analyzed. Nonsynonymous single nucleotide polymorphisms (SNP) were present in eight male patients. None of these individuals showed QT interval prolongation or Brugada-like ECG.

In the *KCNQ1* gene, nonsynonymous changes were not identified. In the *KCNH2* gene, three nonsynonymous changes in three individuals were found: R148W and the in-frame deletion GAG186del are novel, whereas P347S has previously been reported as related to LQTS.[11]

In the *SCN5A* gene, we identified the known common polymorphism H558R with an allelic frequency of 20.46%. A nonsynonymous variant P2006A was found in five unrelated male patients, yielding an allelic frequency of 5.68%. This variant had been described previously as rarely reported in only two of 829 healthy individuals[12] and also in one individual who died due to sudden infant death syndrome.[13] Previously, the P2006A variant has been shown to have a small, yet statistically significant, effect on sodium channel kinetics.

In the *KCNE1* gene, two known common polymorphisms were found: G38S with an allelic frequency of 42.2%, and D85N found in one patient (1.11%). In the *KCNE2* gene, no DNA changes were found.

Coding Variants in Healthy Controls

The full coding sequences of *KCNQ1, KCNH2, SCN5A, KCNE1,* and *KCNE2* genes were analyzed in 90 healthy control individuals. In the *SCN5A* gene, the known common polymorphism H558R

Table II.

Coding Variants in SCD Survivors and CAD Controls

Gene	Exon	Nucleotide Change	Amino Acid Change	Region	Minor Allele Frequency (%) SCD Survivors (N = 45)	Number of Genotypes	Minor Allele Frequency (%) CAD Controls (N = 141)	Number of Genotypes	P
KCNH2	3	C442T	R148W	N-term	1.11	CC = 44, CT = 1, TT = 0	0	CC = 141, CT = 0, TT = 0	–
	4	560_568del9	GAG186del	N-term	1.11	non = 44, non/del = 1, del = 0	0	non = 141, non/del = 0, del = 0	–
	5	C1039T	P347S	N-term	1.11	CC = 44, CT = 1, TT = 0	0	CC = 141, CT = 0, TT = 0	–
SCN5A	28	C6016G	P2006A	C-term	5.6	CC = 40, CG = 5, GG = 0	1.1	CC = 138, CG = 3, GG = 0	0.01

SCD = sudden cardiac death; N-term = N-terminus of the gene; C-term = C-terminus of the gene; CAD = coronary artery disease; P = P-value of probability of case-control difference in allelic frequencies.

was found with an allelic frequency of 27.01%. In the *KCNE1* gene, two common polymorphisms were found: G38S with an allelic frequency of 29.3% and D85N with an allelic frequency of 1.67%. No rare coding variants were found in this group.

Coding Variants in CAD Controls

The exons containing rare coding variants found in VF survivors were sequenced in the control group of 141 patients with CAD without arrhythmias. The *KCNH2* gene variants R148W, GAG186del, and P347S were not present in any of these control samples. The *SCN5A* gene variant P2006A was found in three CAD control individuals, giving an allelic frequency of 1.1%.

Statistics

The allelic frequency of the common polymorphism H558R of the *SCN5A* gene did not differ between the VF survivors (20.46%) and the healthy controls (26.67%, P = 0.319). The same was true for the two previously described common SNPs of the *KCNE1* gene: G38S, which had an allelic frequency of 42.2% in the patients and 29.4% in the healthy controls (P = 0.04), and D85N, which was found in one patient and three controls (P = 0.721).

The carrier frequency of all the rare sequence variants (R148W, GAG186del, P347S in *KCNH2*, P2006A in *SCN5A*) was significantly higher in the VF survivors (8/45, 17.8%) than in the CAD controls (3/141, 2.2%, P = 0.001). Evaluated according to OR, the risk of this genotype in the VF survivors is 8.36 (95% CI 2.13–32.84, P = 0.001).

The allelic frequency of the rare variant P2006A of the *SCN5A* gene was significantly higher among the VF survivors (5.6%) compared with the healthy control subjects (0%, OR 11.13, 95% CI 1.26–98.34, P = 0.003). The same was true for allelic frequencies of this polymorphism in the VF survivors (5.6%) and the CAD control subjects (1.1%, P = 0.01, OR 5.75, 95% CI = 1.32–25.11, P = 0.02). There were no differences in the frequency of this variant between the healthy controls (0%) and the CAD control subjects (1.1%, P = 0.226).

The results are summarized in Tables II–IV.

Discussion

In the present study, we performed a mutation analysis of five cardiac ion-channel genes in VF survivors with CAD to test the hypothesis that ion-channel gene polymorphisms are more common in SCD cases compared with a control population. Our hypothesis was based on findings from previous population studies that demonstrated

Table III.

Coding Variants in SCD Survivors and Healthy Controls

Gene	Exon	Nucleotide Change	Amino Acid Change	Region	Minor Allele Frequency (%) SCD survivors (N = 90)	Number of Genotypes	Minor Allele Frequency (%) Healthy Controls (N = 90)	Number of Genotypes	P
KCNH2	3	C442T	R148W	N-term	1.11	CC = 44, CT = 1, TT = 0	0	CC = 90, CT = 0, TT = 0	0.333
	4	560_568del9	GAG186del	N-term	1.11	non = 44, non/del = 1, del = 0	0	non = 90, non/del = 0, del = 0	–
	5	C1039T*	P347S	N-term	1.11	CC = 44, CT = 1, TT = 0	0	CC = 90, CT = 0, TT = 0	0.333
KCNE1	3	G112A	G38S	Extracellular	42.2	GG = 14, GA = 24, AA = 7	29.4	GG = 41, GA = 41, AA = 5	0.04*
	3	G253A	D85N	Cytoplasmatic	1.11	GG = 44, GA = 1, AA = 0	2	GG = 87, GA = 3, AA = 0	0.721
SCN5A	12	A1673G	H558R	DI-DII	21.11	AA = 26, AG = 19, GG = 0	26.67	AA = 47, AG = 38, GG = 5	0.319
	28	C6016G	P2006A	C-term	5.6	CC = 40, CG = 5, GG = 0	0	CC = 90, CG = 0, GG = 0	0.003

SCD = sudden cardiac death; N-term = N-terminus of the gene; DI-DII = linker of the DI and DII domains of the gene; CAD = coronary artery disease; P = P-value of probability of case-control difference in allelic frequencies. *Not significant after correction on double polymorphism.

a strong genetic component of SCD. One of the pathways by which genetic variation in pathophysiological mechanisms may contribute to increased risk of SCD is electrogenesis and conduction in the myocardium. In our study, the carrier frequency of all the rare sequence variants was significantly higher in the VF survivors (8/45, 17.8%) than in CAD controls (3/141, 2.2%, P = 0.001). In the healthy controls no rare coding variants were found.

Recently, several studies have investigated this issue. The first was conducted as part of the Oregon Sudden Unexpected Death Study.[14] In 67 (18% female) SCD cases and 91 control individuals, mutation analysis of the *SCN5A* gene was performed. Nonsynonymous nucleotide changes were found in 4% of the cases and 1% of the controls (P = 0.31). Other genes were not analyzed. In the most recent study, the authors performed mutation analysis of five cardiac ion-channel genes, *SCN5A*, *KCNQ1*, *KCNH2*, *KCNE1*, and *KCNE2*, in 113 SCD cases included in two large prospective cohorts of women (Nurses' Health Study) and men (Health Professional Follow-Up Study).[15] No mutations or rare variants were identified in any of the 53 male subjects, whereas five rare missense variants were identified in *SCN5A* in six of 60 women (10%). Exons containing these variants were sequenced in 733 control samples from the same population; the overall frequency of these rare variants in *SCN5A* was significantly higher in the SCD cases (6/60, 10.0%) than in controls (12/733, 1.6%, P = 0.001). The other exons were not analyzed in the control individuals included in this study. The conclusion from these studies was that rare variants in ion-channel genes were not common causes of SCD. However, the results might have been influenced

Table IV.
Coding Variants in the Investigated Individuals—Statistic Summary

Gene	Exon	Nucleotide Change	Amino Acid Change	Region	Minor Allele Frequency (%)			P		
					SCD Survivors (N = 45)	CAD Controls (N = 141)	Healthy Controls (N = 90)	SCD vs Healthy	SCD vs CAD	CAD vs Healthy
KCNH2	3	C442T	R148W	N-term	1.11	0	0	NS	NS	–
	4	560_568del9	GAG186del	N-term	1.11	0	0	NS	NS	–
	5	C1039T	P347S	N-term	1.11	0	0	NS	NS	–
SCN5A	28	C6016G	P2006A	C-term	5.6	1.1	0	0,003	0.01	NS

SCD = sudden cardiac death; CAD = coronary artery disease; N-term = N-terminus of the gene; C-term = C-terminus of the gene; P = P-value of probability of case-control difference in allelic frequencies; NS = not significant.

by the fact that in both reports, the initial ECG was not available in a substantial proportion of the investigated individuals. Thus, the cause of death was not necessarily arrhythmic; previous studies have reported that the first recorded rhythm in patients with sudden cardiac arrest is not VF in at least 20%–25% of patients of either sex.[16]

In contrast to the previous two studies, we included only individuals with documented VF. In all patients, CAD was confirmed by coronary angiography. Although we excluded individuals in whom VF was related to acute myocardial infarction, the majority of these VF survivors had suffered a previous myocardial infarction in the past and consequently they had depressed LVEF. In this purely arrhythmic SCD group, the frequency of nonsynonymous DNA changes was significantly higher in the VF survivors compared with the control subjects.

Nonsynonymous DNA change does not necessarily indicate a change in protein function. The presence or absence of such rare variants in control populations does not always predict whether the variant will be associated with a functional alteration in channel properties. In the *SCN5A* gene, the C-terminal P2006A polymorphism was found in five unrelated patients in our study. This variant has been described previously as being rare, reported in only two of 829 healthy individuals[12] and in one infant who died due to sudden infant death syndrome.[13] In that study, the P2006A variant was associated with a small, yet statistically significant, level of increased persistent current combined with depolarizing shifts in voltage dependence of inactivation and more rapid recovery from inactivation. The authors speculated that the level of increased persistent current is not sufficient to evoke arrhythmia and that other factors are probably needed for full pathological expression. This fits well with the concept of repolarization reserve proposed by Roden in 1998: normal hearts include multiple, redundant mechanisms to accomplish normal repolarization. Deterioration of one of these mechanisms will not lead to failure of repolarization and no QT interval changes will be present, but under stress conditions such as CAD and hypokalemia, latent ion-channel mutations can increase the risk of malignant arrhythmias.[17]

Due to technical reasons, it was not possible to perform functional studies of mutated variants of the *KCNH2* channel and a literature search was performed to assess the putative functional effects of the SNPs found in the present study. In the *KCNH2* gene, all three polymorphisms were located in the N-terminal part of the protein. N-terminal residues are instrumental in rapid inactivation and sensor–effector coupling

for channel opening.[18] Nevertheless, in one study deletion of cca 220 amino acids, preserving a cluster of mainly positively charged residues upstream to S1 segment (Δ143–361), induced activation properties that closely resembled the wild-type channel. These results indicate that a large part of the proximal domain is not essential for a wild-type channel activation phenotype.[19] All three changes found in this study were located within that region. On the other hand, N-terminal mutations (including P347S found in our study) have previously been shown to be related to LQTS.[11] Thus, further functional studies are necessary.

To date, in LQTS patients, hundreds of mutations have been reported within the five most common genes, more than half of which are unique, novel mutations. We had anticipated a similar situation in SCD survivors. However, in contrast, in five unrelated patients, the same *SCN5A* variant P2006A was found. A larger study is needed to determine if this variant is a, probably region-specific, risk stratification marker.

The prevalence of ion-channel variants remains largely unknown. Recently, Ackerman et al. published two studies determining the frequency and spectrum of ion-channel variants in approximately 800 apparently healthy individuals.[12,20] Excluding the common polymorphisms, 14%–25% of subjects (according to ethnicity) had at least one nonsynonymous potassium-channel variant and 3%–5.6% of subjects had at least one nonsynonymous sodium-channel variant. The authors commented that these analyses do not represent a population-based genetic epidemiologic study. Another study in Germany included 94 individuals from monozygotic twin pairs and 47 pairs of dizygotic twins,[21] all of whom were healthy. Excluding the common polymorphisms, only two rare nonsynonymous polymorphisms were found in this group. In our group of 90 healthy individuals, no rare coding variants were found. Comparison of these data sets is difficult, also due to probable regional differences.

There are several limitations to our study, the most important of which is the small number of investigated individuals. Sufficient numbers could only be provided by a multicenter study owing to the incidence of SCD events and the capacity of individual centers to perform routine genetic methods. The sensitivity of SSCP is about 90%, thus, some variants might be missed. Due to the small number of women included in the present study, it was not possible to perform any sex-related associations. Missing information on SCD in family history prevents an analysis of familial segregation of the event.

In conclusion, the prevalence of selected, rare coding variants in five long QT genes was significantly higher in cases versus controls, confirming a mechanistic role for these genes among a subgroup of patients with CAD and VF.

Despite the great hopes that were expressed after sequencing of the human genome a decade ago, our understanding of the genetic background of pathophysiological processes remains poor. Recent observations have indicated that genomic architecture is not collinear and that the same sequences are used for multiple independently regulated transcripts and as regulatory regions.[22] The possible SCD susceptibility alleles are likely to be as prevalent in noncoding regions of the genome as in coding sequences.[23] Identification of such alleles will be a task for genome-wide association studies and the new emerging technologies developed for mass DNA sequencing. Any future genetic screening for SCD risk stratification should involve evaluation of large numbers of genomic variants in many pathways, each of which may be relevant to the risk of arrhythmias.

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U pacientů po prodělané arytmické bouři byla provedena mutační analýza genu *RyR2* asociovaného s hereditárním arytmickým syndromem CPVT. Byly u nich nalezeny četné nekódující varianty, jejichž výskyt se ale nelišil při srovnání s kontrolní populací. Role variant genu *RyR2* v patofyziologii náhlé smrti je tedy nejspíše omezený.

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Mutation analysis of RyR2 gene in patients after arrhythmic storm ☆

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ABSTRACT

Introduction: Mutations of RyR2 gene encoding calcium channel of sarcoplazmatic reticulum are the cause of congenital catecholaminergic polymorphic ventricular tachycardia. The aim of this study was to test the hypothesis that RyR2 variants can increase occurrence of malignant arrhythmias in patients with structural heart diseases.
Methods: The investigated group consisted of 36 patients with structural heart diseases with ICD implanted who suffered arrhythmic storm. In the control group there were 141 patients with coronary artery disease who were hospitalized at our department owing to an acute coronary event and they were alive at least 3 years after the index event. Thus, they could be considered as a group with a low risk of sudden cardiac death. In all of them mutation analysis of RyR2 gene was performed.
Results: We detected 16 different sequence changes of RyR2 gene in both groups. None of the found nucleotide polymorphisms led to amino acid changes, were located close to splice sites or had any similarity to known splicing enhancer motifs. The occurrence of these variants was not different in both groups.
Conclusions: The prevalence of RyR2 gene variants was not different in cases versus controls suggesting a limited role of this gene in the arrhythmogenesis in structural heart disease patients.

1. Introduction

The leading cause of cardiovascular mortality is sudden cardiac death (SCD). Estimated incidence of SCD is more than 3 million people in the world per year. It means about 200–400 thousand in the U.S. population per year and in Europe it accounts for about 2500 deaths per day. Nevertheless, the SCD incidence in the general population is low (0.1–0.2% per year, i.e. 1–2 patients/1000 population). Approximately 50% of all coronary heart disease deaths are sudden [1]. In most cases, the underlying cause of death is the rapid onset of lethal ventricular arrhythmias in the setting of acquired heart disease. In our study we considered the common knowledge, that the risk of sudden cardiac death is higher in patients with structural heart disease (it means chronic ischemic heart disease or dilated cardiomyopathy) and with systolic

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Table 1 – Clinical characteristics of investigated individuals.

	Arrhythmic storm ($n = 36$)	CAD controls ($n = 141$)	p
Male/female	33/3	129/12	0,4676
Age (years)	69,4±11,4	66,7± 10,7	0,4610
Left ventricle ejection fraction	32±9%	42± 9%	<0,0001
CAD	69%	100%	<0,0001
History of myocardial infarction	21(58%)	129(90%)	<0,0001
dCMP	11(31%)	0	<0,0001

CAD = coronary artery disease. dCMP = dilated cardiomyopathy.

dysfunction of left ventricle. Data from MADITII study showed that during 3 years only one third of patients with primary preventive implantation of ICD needed the therapy from the device due to malignant arrhythmias. The remaining two thirds of patients were only ICD carriers [2]. Observations from population-based studies demonstrate a strong genetic component of SCD [3–6]. It is clear that there must be genetically determined variations of physiological processes that increase the risk of SCD. It is well known that myocardial ion channel gene mutations lead to increased SCD risk in patients with relatively rare diseases, such as long QT syndrome (LQTS), Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia (CPVT).

Ventricular premature complexes (VPCs) could initiate malignant arrhythmias. High occurrence of VPCs is typical for rare hereditary disease called CPVT. For this disorder stress syncope or sudden cardiac death are typical, manifested in young patients most often before 40 years of age. The mortality is high, almost 50%. Mutations of RyR2 gene are found in about 60% of patients with clinical diagnosis of CPVT [7]. An important fact is that the resting ECG is completely normal. But during stress VPCs become more frequent and can progress in typical bidirectional ventricular tachycardia. In our study we tried to test the hypothesis, that mutation of RyR2 receptor could increase the risk of malignant arrhythmias in patients with structural heart disease.

2. Methods

2.1. *Investigated subjects*

The investigated group consisted of 36 Caucasian patients from the region of south Moravia in the Czech Republic. These individuals were identified from the ICD registry of our department, partially retrospectively (years 1998–2006) and from 2007 new consecutive patients were included prospectively. The inclusion criteria were: (1) history of arrhythmic storm defined as 3 or more sustained ventricular tachyarrhythmias within 24 h detected and treated from ICD. (2) structural heart disease such as dilated cardiomyopathy (dCMP) or CAD verified by echocardiography and coronary angiography.

The control group consisted of 141 patients with CAD who were hospitalized at our department owing to an acute coronary event. For all patients the diagnosis was confirmed by coronary angiography. All patients had depressed left ventricle ejection fraction (LVEF) and they were alive at least 3 years after the index event. Thus, they could be considered as a CAD group with a low risk of SCD. Clinical characteristics of both groups are summarized in Table 1.

Informed consent was obtained from all patients and peripheral blood samples were taken for genomic DNA preparation. The study protocol was approved by Ethical Committee of the University Hospital Brno and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. *Mutation analysis*

Mutation analysis of the RyR2 gene was performed in all members of the arrhythmic storm group. Genomic DNA samples were isolated from peripheral blood lymphocytes and PCR amplified fragments covering hot spot areas of RyR2 exons 2–4, 6–15, 17–20, 39–49, 83, 84, 87–97–105 were analyzed by direct sequencing on the instrument ABI PRISM 3130 (Life Technologies, USA). For mapping of exons 3, 97 and 105 deletions multiplex ligation-dependent probe amplification analysis (MLPA) was used. Detailed methodology has been described elsewhere [8–10]. The regions containing sequence variants found in the arrhythmic storm group were sequenced in the control group of 141 patients with CAD without arrhythmias.

2.3. *Statistics*

Values are given as mean±SD. Demographic data were analyzed by the F test for variance and Student's t-test. The distributions of allelic frequencies and their differences were calculated using χ^2 tests. The program package Statistica v. 8.0 (Statsoft Inc., Tulsa, OK, USA) was used for all statistical analyses.

3. Results

We detected 16 different sequence changes in both groups. Twelve of these DNA changes were already described in the study of Bagattin et al. [11] or in the database Ensemble [12]. The other have not been described yet. None of the found nucleotide polymorphisms led to amino acid changes and were located close to splice sites or had any similarity to known splicing enhancer motifs. The occurrence of these

Table 2 – RyR2 gene variants in investigated # individuals.

Nucleotide change	Exon/intron	Allelic frequency (%)		p	References
		Arrhythmic storm ($n = 36$)	Control group ($n = 141$)		
c.[268−100G>T]	intron 3–4	4,0	6,2	0,5414	–
c.[458−8A>C]	intron 7–8	16,2	12,1	0,2991	12
c.[671−11T>A]	intron 9–10	43,2	53,8	0,1003	12
c.[1000−31T>A]	intron 12–13	1,3	2,7	0,4860	–
c.[1353C>T]	exon 15	33,8	40,5	0,2708	12
c.[6682+7ZT>G]	intron 43–44	36,5	47,0	0,0924	12
c.[6682+93C>A]	intron 43–44	27,0	16,2	0,0487	–
c.[6900T>C]	exon 45	94,5	87,8	0,1117	13
c.[11320−23C>T]	intron 82–83	35,1	29,7	0,4182	12
c.[12957T>C]	intron 89–90	58,1	68,9	0,0928	–
c.[13470+16A>G]	intron 92–93	33,8	28,3	0,4091	12
c.[13470+47G>A]	intron 92–93	36,5	43,2	0,2722	12
c.[13777−21G>A]	intron 94–95	55,4	42,5	0,0479	12
c.[13777−6A>G]	intron 94–95	50,0	36,2	0,0318	12
c.[13907+12A>C]	intron 95–96	59,5	46,0	0,0390	12
c.[14085−(11−22delT)]	intron 97–98	47,3	40,8	0,3231	–

variants was not different in both groups. The results are summarized in Table 2.

4. Discussion

In the present study, we performed a mutation analysis of a cardiac ion-channel gene important in arrhythmogenesis. We test the hypothesis that mutation of RyR2 gene could increase risk of malignant arrhythmias in patients with structural heart diseases. Our hypothesis was based on findings of previous population studies that demonstrated a strong genetic component of SCD.

Recently, several studies have investigated the issue of the influence of genetic variants on the risk of arrhythmias in patients with structural heart diseases. In these studies the prevalence of rare coding variants in so called long QT genes was significantly higher in patients suffering from ventricular fibrillation as compared to control group [13–15].

The RyR2 gene was chosen because of its well known role in the arrhythmogenesis in CPVT. This disease is characterized by high occurrence of VPCs. We hypothesized that such RyR2 mutations can exist which usually remain latent, but under stress conditions, such as structural heart disease, they can increase the risk of ventricular arrhythmias also in normal population.

The RyR2 gene is one of the largest genes in the human genome and its analysis is time consuming. The majority of mutations appear to cluster in three regions of the predicted RyR2 protein topology, and about 65% of published mutations of the RyR2 gene are located in these regions [9,16]. Therefore we used a tiered targeting strategy suggested by groups in the Mayo Clinic and the Netherlands [9]. Although we expected only rare finding of sequence changes of RyR2 gene, the opposite was true. The occurrence of sequence variants was common in both groups. This suggests that these regions are probably rather polymorphic in population. In our study 16 sequence changes of RyR2 gene were detected in both groups. None of the found nucleotide polymorphisms led to amino acid changes, and were located close to splice sites or had any similarity to known splicing enhancer motifs. Nevertheless, there is emerging evidence that even synonymous DNA variants can play important role in protein structure changes. On the other side there were no statistical differences in their allelic frequencies, thus the probability of any functional effect of these variants is low.

The most important limitation of our study is the small number of investigated individuals. Although the control group was age and sex matched and all the control individuals have depressed left ventricular systolic function and coronary artery disease confirmed by angiography, there were differences in ejection fraction and the etiology of heart failure. This could have possible impact on the results. Nevertheless, the prevalence of DNA variants was high also in the control group. Using the tiered targeting strategy of RyR2 gene mutation analysis can have an impact on the robustness of the estimate of the prevalence of RyR2 mutations among the cohort. Due to the small number of women included in the present study, it was not possible to perform any sex-related associations.

5. Conclusions

The prevalence of RyR2 gene variants was not different in cases versus controls suggesting a limited role of this gene in the arrhythmogenesis in structural heart disease patients.

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Vycházejíce s našeho předchozího nálezu signifikantně vyššího výskytu kódujících variant genů pro iontové kanály myokardu u pacientů s ICHS po prodělané fibrilaci komor, jsme ve stejném souboru analyzovali i výskyt variant v promotorech stejných genů. Zjistili jsme, že varianty promotorů jsou běžné jak u pacientů, tak v kontrolním souboru. Srovnání s mezinárodními databázemi naznačuje významné geografické odlišnosti ve výskytu variant promotorů.

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Original Research Article

Mutation analysis of ion channel genes promoters in ventricular fibrillation survivors with coronary artery disease

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ABSTRACT

Introduction: Strong evidence suggests that sudden cardiac death (SCD) is genetically determined. In our previous study we found that the prevalence of selected, rare coding variants in 5 long QT genes was significantly higher in ventricular fibrillation (VF) survivors with coronary artery disease (CAD) than in controls. In the present study we performed mutational analysis of the promoters of 5 LQTS-related myocardial ion channel genes in the same group of patients and in control populations.

Methods: The promoters of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2* genes were analyzed in 45 CAD individuals – survivors of documented VF. The allelic frequencies were compared either to data from the 1000 Genomes Project or from a local DNA bank of patients with coronary artery disease and no malignant arrhythmia (141 individuals).

Results: In 34 (75.5%) of 45 VF survivors 9 different promoter variants were found: 2 in *KCNQ1* gene promoter, 1 in *KCNE1* promoter, and 6 in *SCN5A* promoter. Statistically significant differences were found in the allelic frequencies of both *KCNQ1* gene promoter variants: 1-182C>T ($P=0.008$), 1-119G>A ($P=0.007$). Nevertheless, these variants did not segregate with long QT phenotype in a previous study. While the allelic frequency of the *SCN5A* gene promoter variant 225-1072T>C significantly differed in VF survivors compared to the 1000 Genomes Project ($P=0.001$), this allelic frequency was not different when compared to the group of local CAD controls.

Conclusions: Our findings demonstrated that variants of ion channel gene promoters are common, both in VF survivors and control groups. These results suggest that promoter variants are geographically-specific and are not a common cause of SCD.

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1. Introduction

There is strong evidence that sudden cardiac death (SCD) is genetically determined [1–4]. In our previous study we found that the prevalence of selected, rare coding variants in 5 long QT genes was significantly higher in cases compared to controls, confirming a mechanistic role for these genes in a subgroup of patients with coronary artery disease (CAD) who had suffered ventricular fibrillation (VF) [5].

Ten years after human genome sequencing it has become clear that coding sequences represent only about 1.5% of human genome sequence [6]. The remaining portion is either so called "DNA junk" or, more likely, contains important regulatory sequences.

In the present study we performed mutational analysis of the non-coding parts of genes – the promoters – of 5 LQTS-related myocardial ion channel genes in patients with CAD who had suffered documented VF at the time of SCD event and in control population.

2. Methods

2.1. *Investigated subjects*

The group of patients was identical to our previous study [5], consisting of 45 Caucasian patients (5 females, 40 males) from the region of south Moravia in the Czech Republic (population approximately 1.5 million). The primary inclusion criterion was VF documented at the time of circulatory arrest not related to acute phase of myocardial infarction (i.e. more than 48 h after index event). Such individuals were identified from ICD registry of our department, partially retrospectively (years 1998–2006), from 2007 new consecutive patients were included prospectively. Two patients suffered VF while staying in a hospital for more than 72 h after acute myocardial infarction. All other individuals suffered circulatory arrest in the out-of-hospital setting – VF was recorded by emergency car staff. Patients were successfully resuscitated and then transferred to our department, either directly or via regional hospitals. After circulatory stabilization and improvement in mental status, all patients underwent comprehensive cardiological examination. None of the patients had an acute myocardial infarction at the time of arrhythmia according to electrocardiogram (ECG) and serum troponin level analysis. In all patients coronary artery disease was confirmed by coronary angiography findings of significant stenoses (>70%) or chronic occlusions. The left ventricle ejection fraction (LVEF) was calculated from echocardiography analysis. In three patients, QT interval assessment was not possible due to left bundle branch block or atrial fibrillation. None of the other 45 patients showed substantial QT interval prolongation or Brugada-like ECG.

As a control, we used data from the 1000 Genomes Project, which sequences the genomes of a large number of people, to provide a comprehensive resource on human genetic variation. To date, the database contains data on genetic variation in 1092 human genomes from various populations [7].

In cases where the data from the 1000 Genomes Project were not available, another control group was used, consisting of 141 patients with CAD who were hospitalized at our department owing to an acute coronary event. For all patients, the diagnosis was confirmed by coronary angiography. All patients had depressed LVEF and were alive at least 4 years after the index event and, accordingly, they could be considered as a CAD group with a low risk of SCD. Clinical characteristics of both groups of patients are summarized in Table 1.

Informed consent was obtained from all individuals and peripheral blood samples were taken for genomic DNA preparation. The study protocol was approved by the Ethical Committee of the University Hospital Brno and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. *Genomic DNA samples and polymerase chain reaction amplification*

The genomic DNA of all cases was analyzed for mutations in the promoter sequences of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2* genes. Genomic DNA was extracted from peripheral blood samples according to a standard protocol using DNA BloodSpin Kit and the classical method of ethanol progression.

The promoter sequences of *KCNQ1*, *KCNH2* and *KCNE1* genes (GenBank accession nos. **NG008935.1**, NG008916.1, NG009091.1), were amplified by multiplex polymerase chain reaction (PCR). The following primer pairs were designed and used: 7 primer pairs for *KCNQ1* promoter, 6 primer pairs for *KCNH2* promoter and 1 primer pair for *KCNE1* promoter. The PCR amplifications were performed using HotStarTaq Master Mix (Qiagen, Inc., Valencia, CA, USA). Each PCR was performed in 200-μl thin-walled PCR tubes in a total reaction volume of 25 μl using Verity Thermal Cycler (Applied Biosystem, Foster City, CA, USA).

A total of 12 oligonucleotide primer pairs was designed and used to amplify the promoter area of the *SCN5A* gene (GenBank accession no. AY313163). The core promoter

Table 1 – Clinical characteristics of investigated individuals.

	VF survivors ($n=45$)	CAD controls ($n=141$)	p
Male/female	40/5	129/12	0.8181
Age	68.6±9,5	66.7±10.7	0.2885
Left ventricle ejection fraction	40±12%	42±9%	0.35238
History of myocardial infarction	32 (71%)	127 (90%)	0.2718

VF – ventricular fibrillation, CAD – coronary artery disease.

sequence of *KCNE2* gene was predicted to correspond to base pairs 7481–7746 of sequence DQ784804 using the Web Promoter Scan Service program, version 1.7. This region was amplified using one oligonucleotide primer pair. PCR amplifications were performed using Taq DNA polymerase (Fermentas, Inc., Glen Burnie, MD, USA) and HotStarTaq Master Mix (Qiagen, Inc., Valencia, CA, USA). Each PCR was performed in 200-μl thin-walled PCR tubes in a total reaction volume of 25 μl. Each PCR amplification was performed using a Sensoquest Labcycler (Progen Scientific, Ltd., Mexborough, UK) and a Perkin Elmer 2400 machine (Applied Biosystem, Foster City, CA, USA).

2.3. DNA sequencing

DNA sequence analysis was carried out in an ABI 3130 (Applied Biosystem, Foster City, CA, USA) to screen for *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE2* and *KCNE1* promoter sequence variations. Sequencing analyses were performed using forward and reverse primers.

For purification of the amplified samples, the MinElute PCR Purification Kit (Qiagen, Inc., Valencia, CA, USA) was used. Cycle sequencing was performed using the Big Dye Terminator Kit (Applied Biosystems, Foster City, CA, USA) and the DyeEx2.0 Spin Kit (Qiagen, Inc., Valencia, CA, USA) was used for purification of samples after cycle sequencing.

The resulting sequences of the screened *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE2*, and *KCNE1*, gene promoters were aligned with the wild-type sequences deposited in NCBI (GenBank accession nos. NG008935.1, NG008916.1, **NG009091.1**, **AY313163**, **DQ784804**). The numbering was based on Yang et al. [8] and the allelic frequencies were compared to data from the 1000 Genomes Project, if available [7]. Otherwise the regions containing sequence variants found in VF survivors were sequenced in the control group of 141 patients with CAD without arrhythmias (described above).

2.4. Statistics

The distributions of allelic frequencies and their differences were calculated using χ^2 tests. Odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the risks associated with the detected polymorphisms. To calculate the significance of OR, Fisher's exact test was used. The program package Statistica v. 8.0 (Statsoft Inc., Tulsa, OK, USA) was used for all statistical analyses.

3. Results

In 34 (75.5%) of 45 VF survivors 9 different DNA sequence variants were found: 2 in *KCNQ1* gene promoter, 1 in *KCNE1* promoter, and 6 in *SCN5A* promoter. No variants were detected in the promoters of *KCNH2* and *KCNE2* genes. Variants in more than one gene promoter were present in 6 patients, and multiple variants in single gene promoter were found in 16 patients.

3.1. *KCNQ1* gene promoter variants

In *KCNQ1* gene promoter variants 1-182C>T and c. 1-119G>A were found in 15 and 3 patients, respectively. The c. 1-119G>A polymorphism segregated with the c. 1-182C>T in all 3 cases. These variants were present in the 1000 Genomes Project data.

3.2. *KCNE1* gene promoter variant

A single variant, c. 1-107insG, was found in the *KCNE1* gene promoter in 3 VF survivors. Data on this particular variant were not available from then 1000 Genomes Project, and its allelic frequency was tested in the CAD control group.

3.3. *SCN5A* gene promoter variants

Six promoter variants were found in the *SCN5A* gene in 22 VF survivors. More than one *SCN5A* promoter variant was present in 13 VF survivors. The following variants have been described in the 1000 Genomes Project previously: c. -225-2038G>T, c. -225-1823C>T, c. -225-834T>C, c. -225-1744G>C, c. -225-1072T>C. For the c. -225-775T>A variant, no data were available from the 1000 Genomes Project and, therefore, its allelic frequency was tested in the CAD control group.

3.4. Statistic results

The allelic frequencies of all promoter variants in the cases and control groups are summarized in Table 2. Statistically significant differences were found in the allelic frequencies of both *KCNQ1* gene promoter variants: c. 1-182C>T (OR=2.57, CI=1.18–5.57 for the C allele in VF survivors compared to the CAD group, P=0.008) and c. 1-119G>A (OR=7.76, 95% CI=1.06–56.77 for the G allele in VF survivors compared to the control group, P=0.007). The allelic frequency of *SCN5A* gene promoter variant c. 225-1072T>C was significantly different in VF survivors compared to the S1000 Genomes Project (OR=7.28, 95% CI=1.00–53.28 for the T allele in VF survivors compared to the control group, P=0.001), but was not different between the cases and the group of local CAD controls.

4. Discussion

In the present study, we performed a mutation analysis of promoters of five cardiac ion channel genes in VF survivors with CAD to test the hypothesis that polymorphisms in these regions are more common in VF survivors compared with a control population. This study was an extension of our previous study in which we found that the carrier frequency of rare coding variants of these genes was significantly higher in the VF survivors (8/45, 17.8%) than in CAD controls (3/141, 2.2%, P=0.001). In the promoter regions of the same genes we found 9 different DNA sequence variants. The allelic frequencies of three of these variants were significantly different compared to data from 1000 Genomes Project, which was used as a primary control due its statistical power to detect

Table 2 – Allelic frequencies of promoter variants.

Gene	Variant	VF survivors (n=45)	1000 genomes	CAD controls (n=141)	P
KCNQ1	c. 1-182C>T	C=0.823 T=0.177	C=0.643 T=0.357	–	0.008
	c. 1-119G>A	G=0.967 A=0.033	G=0.850 A=0.150	–	0.007
SCN5A	c. -225-2038G>T	G=0.86 T=0.14	G=0.864 T=0.136	–	NS
	c. -225-1823C>T	C=0.87 T=0.13	C=0.859 T=0.141	–	NS
	c. -225-1744G>C	G=0.97 C=0.03	G=0.993 C=0.007	–	NS
	c. -225-1072T>C	C=0 T=1	C=0.142* T=0.86	C=0.003+ T=0.997	*0.001 +NS
	c. -225-834T>C	T=0.87 C=0.13	T=0.883 C=0.117	–	NS
	c. -225-775T>A	T=0.98 A=0.02	–	T=1 A=0	NS
KCNE1	c. 1-107insG	Allele=0.956 insG=0.044	–	Allele=0.933 insG=0.067	NS

VF – ventricular fibrillation, CAD – coronary artery disease, P=P-value of probability of case-control difference in allelic frequencies, NS – not significant.
* - VF survivors vs. 1000 Genomes,
+ - VF survivors vs. CAD controls

most genetic variants with frequencies of at least 1%. A key goal of the 1000 Genomes Project is to identify more than 95% of single nucleotide polymorphisms at 1% frequency in a broad set of populations and to date it contains data on genetic variation from 1092 human genomes in 14 populations drawn from Europe, East Asia, sub-Saharan Africa and the Americas [7].

Data on the prevalence of ion channel promoter polymorphisms in arrhythmic patients are limited [9]. The variants we detected in KCNQ1 gene promoter were also present in the 1000 Genome Project dataset and the occurrences of both polymorphisms in this promoter were significantly different in VF survivors group compared to the 1000 Genomes Project dataset. Data concerning the possible functional effect of these polymorphisms are lacking; in our previous work these variants were found also in patients with clinical diagnosis of long QT syndrome (LQTS) but in no case did the particular variant segregate with LQTS phenotype [10]. Thus, any possibility of their significant functional effect is very limited.

The variant we detected in the KCNE1 gene promoter has not been described previously [11], and its allelic frequency did not differ between VF survivors and CAD patients without malignant arrhythmias.

The SCN5A gene promoter is highly polymorphic, reflected in the fact that in this study we identified 6 different polymorphisms in this promoter. With the exception of c. -225-1072T>C all these variants were the subject of a study by Yang et al. [8], which found that study only promoter activity in c. -225-775 T>A variant was significantly reduced in cardiomyocytes. Such variant may modulate sodium-channel-related physiology in the face of environmental stressors such as transient myocardial ischemia. Nevertheless, allelic frequency of this variant did not differ between VF survivors and CAD group in our study, indicating that the potential for a significant functional effect is limited.

No functional data are available for the variant c. -225-1072T>C. While the allelic frequencies of this variant were significantly different in VF survivors compared to the 1000 Genomes Project dataset, its allelic frequency in VF survivors was not different when compared to the group of local CAD controls. These results indicate that this variant may be a regionally specific, and likely to be a non-functional polymorphism.

5. Limitations

There are several limitations to our study, the most important of which is the small number of investigated individuals. Owing to the incidence of SCD events and the capacity of individual centers to perform routine genetic methods, larger numbers could only be provided by a multicenter study. Due to the small number of women included in the present study it was not possible to perform any sex-related associations. Allelic frequencies of variants found in VF survivors were compared with data from the 1000 Genomes Project, which represents a non-specific population. In the case of KCNQ variants, the missing functional studies were substituted by data from genotype–phenotype analyses in LQTS subjects.

6. Conclusion

While variants of ion channel genes promoters are common both in SCD individuals and control groups, our results suggest that promoter variants of ion channel genes are not a common cause of SCD.

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V této práci byly výsledky získané v předchozích projektech využity k porovnání parametrů nové generace sekvenátorů („next generation sequencing“) a to v rámci mezinárodní spolupráce.

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PLOS ONE

Towards Clinical Molecular Diagnosis of Inherited Cardiac Conditions: A Comparison of Bench-Top Genome DNA Sequencers

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Abstract

Background: Molecular genetic testing is recommended for diagnosis of inherited cardiac disease, to guide prognosis and treatment, but access is often limited by cost and availability. Recently introduced high-throughput bench-top DNA sequencing platforms have the potential to overcome these limitations.

Methodology/Principal Findings: We evaluated two next-generation sequencing (NGS) platforms for molecular diagnostics. The protein-coding regions of six genes associated with inherited arrhythmia syndromes were amplified from 15 human samples using parallelised multiplex PCR (Access Array, Fluidigm), and sequenced on the MiSeq (Illumina) and Ion Torrent PGM (Life Technologies). Overall, 97.9% of the target was sequenced adequately for variant calling on the MiSeq, and 96.8% on the Ion Torrent PGM. Regions missed tended to be of high GC-content, and most were problematic for both platforms. Variant calling was assessed using 107 variants detected using Sanger sequencing: within adequately sequenced regions, variant calling on both platforms was highly accurate (Sensitivity: MiSeq 100%, PGM 99.1%. Positive predictive value: MiSeq 95.9%, PGM 95.5%). At the time of the study the Ion Torrent PGM had a lower capital cost and individual runs were cheaper and faster. The MiSeq had a higher capacity (requiring fewer runs), with reduced hands-on time and simpler laboratory workflows. Both provide significant cost and time savings over conventional methods, even allowing for adjunct Sanger sequencing to validate findings and sequence exons missed by NGS.

Conclusions/Significance: MiSeq and Ion Torrent PGM both provide accurate variant detection as part of a PCR-based molecular diagnostic workflow, and provide alternative platforms for molecular diagnosis of inherited cardiac conditions. Though there were performance differences at this throughput, platforms differed primarily in terms of cost, scalability, protocol stability and ease of use. Compared with current molecular genetic diagnostic tests for inherited cardiac arrhythmias, these NGS approaches are faster, less expensive, and yet more comprehensive.

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Introduction

Molecular diagnostics are recommended in the management of inherited diseases, for diagnosis and stratified therapy [1,2,3,4], but in practice are under-used due to issues of cost, time and availability of services. Next Generation Sequencing (NGS) DNA analysis technologies have the potential to overcome these issues [5]. Inherited cardiac conditions (ICC), such as inherited arrhythmia syndromes and cardiomyopathies, have been identified as a suitable area to pilot the development of NGS assays for clinical use [6,7]. This is due to the relatively high burden of disease in the population and limitations of current diagnostic approaches in genetically heterogeneous conditions such as these.

A number of bench-top NGS platforms have recently been introduced capable of Gigabase-scale DNA sequencing with relatively short run times (<27 hrs), including the MiSeq (Illumina) and the Ion Torrent Personal Genome Machine (PGM; Life Technologies). Initial studies have used these to characterise genetic targets of clinical significance including; bacterial genomes [8,9,10,11,12,13,14,15,16], the human breast cancer *BRCA* gene [11,17], the cystic fibrosis *CFTR* gene [18], HLA type [19] and somatic variation in cancer [20]. The high

analytical throughput and relative speed make NGS assays very attractive for early clinical implementation, requiring an in-depth understanding of the strengths and limitations of each platform in a clinical diagnostic setting.

A recent study by Loman *et al* [10] compared bench-top NGS platforms for sequencing *E.coli* genomes, which have a GC-content of 50%, during an outbreak investigation. They identified a higher rate of homopolymer-associated indel errors in raw reads when comparing the Ion Torrent PGM to the MiSeq (1.5 and <0.001 errors per 100 bases, respectively). The MiSeq also detected fewer single-base substitutions than the Ion Torrent PGM. A further recent study by Quail *et al* [8] performed similar analyses using a number of different bacterial reference genomes representing a range of GC-contents, including the *B.pertussis* genome which has a GC-content of ~68% with some sub-genomic regions >90%. They observed a higher substitution error rate when using Ion Torrent PGM than the MiSeq platform (1.78 and 0.4 errors per 100 bases, respectively). Again, they reported fewer homopolymer-associated errors in MiSeq data than the Ion Torrent PGM. More variants were called using the Ion Torrent PGM versus MiSeq; however, this resulted in a slight increase in the number of false positive calls using the Ion Torrent PGM platform. Both NGS platforms generated adequate coverage across templates even in sub-genomic regions of very high GC-content. Significant efforts to improve sequencing performance and bioinformatics processing have been undertaken both by the bench-top sequencer manufacturers and the NGS community.

In this study, we used microfluidic multiplex PCR and NGS to sequence six genes that cause inherited arrhythmia syndromes in a panel of well characterised patient-derived genomic DNAs. We compared the performance of two bench-top MiSeq and Ion Torrent PGM DNA sequencing platforms, aiming to develop a comprehensive pipeline applicable to clinical diagnostics.

Materials and Methods

Human Specimens

The Hammersmith and Queen Charlotte's & Chelsea Research Ethics Committee approved the study. DNA was obtained from subjects who had given written informed consent and was provided in accordance with Human Tissue Act, UK guidelines. Fifteen anonymised DNA samples were selected for technical assay evaluation. Eleven (group I) had undergone mutation scanning of five Long QT syndrome (LQT) associated genes (See Table 1) using denaturing high performance liquid chromatography (dHPLC) [21] coupled with Sanger DNA sequence analysis to confirm putative variants. Four (group II) underwent exon PCR amplification and direct Sanger DNA sequence analysis of the full coding sequence of the same five genes.

Target Enrichment by PCR Capture

Initial Access Array primer design was undertaken by Fluidigm Corp. (South San Francisco, CA) using the Primer3 oligonucleotide design tool [22]. Prior to this study the assay was further optimized in-house, with additional primers designed to target regions that were not well captured in pilot studies using the Ion Torrent PGM [23]. In the final assay 386 amplicons targeted the protein-coding sequence of six inherited arrhythmia genes (Table 1), with an overhang at exon boundaries to capture splice site variants. Figure S1 in the Supporting Information illustrates the GC-content and length distribution of the 386 Access Array amplicons.

Genomic DNA templates were amplified using the 48.480 Access Array IFC, according to the manufacturer's instructions

Table 1. Characteristics of six genes included in the assay.

Gene symbol	Gene product	Genomic locus	[1]Reference sequence	Number of exons	Coding cDNA length (bp)	Number of amplicons	Disease association
KCNQ1	I_{Ks} channel, alpha subunit	11p15.5	LRG_285_t1	16	2015	30	LQT1
KCNH2	I_{Kr} channel, alpha subunit	7q36.1	LRG_288_t1	15	3465	54	LQT2
SCN5A	$Na_v1.5$ channel, alpha subunit	3p21	LRG_289_t1	27	6024	78	LQT3
KCNE1	I_{Ks} channel, beta subunit	21q22.12	LRG_290_t1	1	389	4	LQT5
KCNE2	I_{Kr} channel, beta subunit	21q22.12	LRG_291_t1	1	371	5	LQT6
RYR2	Sarcoplasmic reticulum calcium-induced calcium release	1q43	LRG_402_t1	104	14,785	215	CPVT
Total				164	27,049	386	

LRG: locus reference genomic (http://www.lrg-sequence.org); LQT: Long QT syndrome, CPVT: catecholaminergic polymorphic ventricular tachycardia.

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(Fluidigm). In brief, each sample DNA was combined with primer pairs in a microfluidic chip, with a maximum capacity of 48 samples×48 10-plex reactions. The chip was loaded with PCR reagents and transferred to a thermocycler. Common flanking sequences (CS) on each primer pair permit attachment of platform-specific barcode indexes and sequencing adaptors in a subsequent fusion PCR. Pooled amplicons from each DNA template were harvested and used as input for platform-specific library preparation.

Platform-specific Barcode/Adapter Attachment

For MiSeq, we followed standard Fluidigm protocols. Amplicons were diluted 1:100 and subjected to a single fusion PCR reaction using the bidirectional 386 barcode kit, with the FastStart High Fidelity Enzyme kit (Roche), as per manufacturer's instructions. A unidirectional library was prepared for paired-end sequencing: for each reaction, 1 µl of the diluted harvested PCR pool was mixed with forward "A" barcodes (indexes 1 to 15, final concentration 400 nM) and 15µl of PCR pre-mix. Cycling conditions were as follows: initial incubation at 95°C for 10 min; 15 cycles of 95°C for 15 sec, 60°C for 30 sec and 72°C for 1 min; final incubation at 72°C for 3 min; hold at 4°C.

For Ion Torrent PGM, commercial barcoding protocols were not available at the time of the study, so we employed an equivalent fusion PCR approach using custom oligonucleotides, yielding a 10 base pair (bp) barcode and Ion Torrent PGM adaptor (Table S1 in the Supporting Information). The amplicon harvest volume was adjusted to 20µl using PCR certified water, and two barcode-fusion PCR reactions were prepared using opposing CS-tagged primer pairs (e.g. pairing A_BC6_CS1 with CS2_P1, and A_BC6_CS2 with CS1_P1). This strategy permitted sequencing of each amplicon in both orientations, *in lieu* of paired-end sequencing. For each reaction, 10 µl of the Fluidigm harvest was added to 86 µl of a Herculase II Fusion PCR mix, as per manufacturer's instructions (Agilent Technologies Inc, Santa Clara, CA) along with 20 pmol each primer. Cycling conditions were as follows: initial incubation at 98°C for 30 sec; two cycles of 98°C for 30 sec, 54°C for 30 sec and 72°C for 30 sec; final incubation at 72°C for 2 min; hold at 4°C.

MiSeq Sequencing

MiSeq sequencing was performed at the MRC Clinical Sciences Centre Genomics Laboratory, Imperial College London, using MiSeq Reagent kit v1, MCS v1.1.1 and RTA v1.13.56 for performing image analysis, base calling and quality control (QC).

Ion Torrent PGM Sequencing

Ion torrent PGM sequencing was completed at Royal Brompton Hospital using Ion One Touch 200 reagents kits (Release: 20 February 2012, Rev. C), Ion PGM 200 Sequencing Kit (Release: 21 February 2012, Rev. B) and 316 scale chips. Sequence analysis was completed with Ion Torrent Suite 2.2 (ITS2.2; Life Technologies) packages. Sequence analysis and variant calling were subsequently repeated using ITS3.2, but the results were unchanged, and data from ITS2.2 is presented here.

Bioinformatic Primer Trimming and Read Mapping

Default parameters were used for all data processing and analysis stages unless otherwise specified. FastQC version 0.9.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to assess sequence quality metrics for each sample, including per-base and per-sequence quality scores, GC-content, and read length distribution. Raw sequences generated by MiSeq and Ion Torrent PGM included primer sequences at both 5′ and 3′ ends. For MiSeq data, primers were trimmed using an in-house Perl script, before quality control (average base quality in a 30 bp sliding window >20; 3′ read trimming of bases with a quality score <6; removal of reads <20 bp in length) and alignment with BWA (version 0.6.1-r112-master) [24]. Figure S2 and S3 in the Supporting Information demonstrates the base quality and length distribution before and after primer trimming for one sample from MiSeq. Ion Torrent PGM reads were aligned using ITS2.2, incorporating tmap (version 0.3.7). The variantCaller plugin module trimmed primers using an aligned bam file intersected with an amplicon-only bed file. Human genome reference sequence (hg19) was used for both platforms.

Coverage of the target was assessed using BedTools [25]. The number of bases covered at sufficient depth and quality for variant calling was assessed using the Genome Analysis Toolkit (GATK; version 1.5) [26] Callable Loci Walker. Evenness was calculated according to the method described by Mokry *et al* [27] and implemented with the R statistical package (http://www.r-project.org). This yielded a score in the range 0–1, with 1 indicating uniform coverage. Target enrichment factor (EF) was calculated as, $EF = \frac{R/N}{T/G}$, here R represents the reads on target; N represents total mapped reads; T represents target size and G represents genome size [28].

Variant Detection

MiSeq reads were processed using Picard tools (version 1.65, http://picard.sourceforge.net) and Samtools (version 0.1.18) [29], and variants were called with GATK. A standard GATK pipeline was applied including realignment around known indels (dbSNP135) and recalibration. All reads were used for variant calling, without downsampling or removal of PCR duplicates. Variants with QD <5 or MQ <30 or DP<30 were filtered out. For the Ion Torrent PGM, variants were called using the ITS2.2 variantCaller plugin with the Ampliseq and germline workflow. Primers were trimmed and variants called with a variant frequency threshold at 25%. The Integrative Genomics Viewer (IGV) [30] was used for visualization.

Reference Comparator by Sanger DNA Sequencing

Direct dideoxy Sanger DNA sequencing was used to sequence all protein coding regions of five LQT genes in samples from group II. Amplicons were prepared using Platinum *Taq* PCR (Life Technologies) and GC-Rich PCR system (Roche), and sequenced using the ABI 3730XL DNA analyzer (Life Technologies). Though sequenced by NGS, *RYR2* was not included in comparisons as its large size made validation prohibitive. DNA sequence analysis was performed using Sequencher 4.10.1 (Genecodes Inc, Ann Arbor, MI). Any discordant variant calls between NGS and dHPLC in group I were also confirmed by Sanger sequencing. The total number of bases sequenced by the direct Sanger DNA sequencing method was 61,380 bp.

The sensitivity and positive predictive value (PPV) of variant detection were calculated by comparing the gold-standard Sanger data to the NGS data for each platform. 95% confidence intervals (CI) were calculated using Jeffreys interval, implemented in the *binom* package in R.

Results

Sequencing Data Output and Quality

Total sequencing output and mean read lengths from the two approaches were comparable (Table 2). A single MiSeq run

produced 8.13 million reads (1230 Mb of sequence) as compared to three 316 chip-scale Ion Torrent PGM runs that generated 6.56 million reads (1001 Mb of sequence). Raw reads generated by MiSeq have 151 bp fixed length (paired-end read), whilst reads generated by Ion Torrent PGM had a variable length, using the 200 bp Ion Torrent PGM chemistry kits. The average length of reads in three Ion Torrent PGM runs was 150 bp (single-end read).

The two platforms produced a similar yield of filtered sequence bases. The MiSeq platform produced 95.8% high quality (Q20) bases and the Ion Torrent PGM 67.5%. As platforms use different algorithms to estimate base quality, apply different downstream quality filters and call variants differently [31,32], these raw quality scores are most useful for comparing runs within platform, and are provided here for comparison to other datasets. We do not use these data to compare sequencing performance between platforms. For the MiSeq, raw reads contained primer sequences and lower quality bases at the 3′ ends. Primer trimming and quality control discarded 4.6% of reads, and excluded 27.6% of bases; only 18.4% of Q20 bases were excluded, and final trimmed reads comprised 95.8% Q20 bases. 90.7% of the trimmed reads mapped to the reference genome, and 96.7% of these mapped reads were on-target. The average depth of coverage on-target was 1529-fold (Table 3), with evenness 0.68 and EF 110111.

For the Ion Torrent PGM, reads still contained primer sequences after de-multiplexing, thus primer trimming was performed following the alignment procedure. We observed 93.5% raw reads were mapped to the reference genome; 91.2% of the mapped reads were on-target. The average depth of coverage on-target was 1231-fold across all samples (Table 3) with evenness 0.79 and EF 104915. Both evenness and enrichment factor differed significantly between platforms (p-value $<2.2\times10^{-16}$ for evenness and p-value$<3.3\times10^{-6}$ for EF; paired t-test).

Target Enrichment Performance

Figure 1 summarises the coverage of our genes of interest for each platform. Overall, 98.8% of the target region was covered by at least one read for the MiSeq and 98.0% for the Ion Torrent PGM (Table 4 and Figure S4 in Supporting Information). For three genes (*SCN5A1*, *KCNE2* and *RYR2*) coverage consistently approached 100% on both platforms. *KCNQ1* and *KCNH2* were less consistently well covered, averaging 96.2% and 94.1% for MiSeq, and 93.1% and 88.9% for Ion Torrent PGM, respectively. While coverage at 1× was almost complete for *KCNE1*, part of the gene achieved consistently low sequencing depth on the MiSeq only (see Discussion).

The mean coverage of the protein-coding region of every gene was consistently >200 reads on both sequencing platforms (Fig. 1b). The depth of coverage was more consistent between samples on the MiSeq than on the Ion Torrent PGM (Figure 1b). By contrast, within-sample coverage was more consistent on the Ion Torrent PGM (evenness 0.78 vs. 0.68, $p<2.2\times10^{-16}$; Table 3). While the MiSeq provided deeper coverage overall, *KCNE1* was an outlier on this platform (Figure 1b), suggesting a platform-specific sequencing difficulty (See Discussion).

The influence of GC-content on performance was assessed. GC-content was calculated using a 50 bp sliding window and plotted alongside sequencing depth across the target for each NGS platform (*KCNQ1* and *KCNH2* are shown in Figure 2, remaining genes in Supporting Information Figure S5). We found that both platforms performed less well in regions of very high GC-content (*KCNQ1* exons 1 & 8, *KCNH2* exons 1, 4, 12, and the 3′ portion of exon 2). The relationship between GC-content and performance was most reproducible for the Ion Torrent PGM. While the MiSeq displayed more variability in sequencing depth (See Table 3), the relationship with GC was weaker suggesting that other factors may be limiting (Figure S6 in the Supporting Information).

Table 2. Comparison of bench-top NGS platforms.

	MiSeq	Ion Torrent PGM*
NGS runs	1	3
Template preparation	1 hr	3×5.5 hr
Run time	27 hr	3×3 hr
Barcodes	15 (commercial)	3×5 (custom)
Theoretical sequencing output	1.5 Gb	3×1.27 Gb
Actual sequencing output	1.23 Gb	1.00 Gb
Number of sequencing reads	8.13 M	6.56 M
Read length output	151	150**
Paired-end reads	Yes	No
Instrument cost	$125k	$75k
Sequencing cost for assay	$959	3x$686
Per specimen sequencing cost	$64	$137

*316 scale chip; ** average.
doi:10.1371/journal.pone.0067744.t002

Variant Detection

Variant detection was assessed using a panel of variants previously identified by dHPLC mutation scanning (group I) or Sanger sequencing (group II) (Table 5). The majority of known variants were detected on both platforms, with a small number of variants missed by each. NGS platforms also detected a number of variants not previously identified, mainly in samples where dHPLC rather than Sanger sequencing was used for initial variant detection. In these samples, validation by Sanger sequencing confirmed 28/32 (87.5%) unexpected MiSeq variants, and 33/36 (91.7%) Ion Torrent PGM variants (Table 6), which were therefore dHPLC false negatives. The MiSeq produced four genuine false positive (FP) SNP calls, and the Ion Torrent PGM five FPs (four SNPs and one indel), equivalent to positive predictive values (PPV) of 95.9% (MiSeq; 95% CI 90.5–98.6%) and 95.4% (PGM; 95% CI 90.3–98.2%).

Variants not detected by NGS were primarily located in regions without any sequencing coverage. On the MiSeq platform, 14 known variants were not detected (see Table S2 in the Supporting Information). These included a single common polymorphism in *KCNE1* that was present in 12 samples (chr21:35821821, Supporting Information Figure S4), and a separate SNP in the same gene (chr21:35821795). This single exon gene was well covered by the Ion Torrent PGM, but consistently inadequately sequenced by the MiSeq across samples, suggesting a platform-specific, sequence context dependent limitation, rather than a failure of the upstream PCR capture. The final false negative on the MiSeq was also missed by the Ion Torrent PGM (chr7:150645534, *KCNH2*) in same sample, with no sequencing reads on either platform at this region of high GC content (>70%), suggesting that the upstream PCR did not capture this region. The final variant missed by the Ion Torrent PGM was found in a well-captured region of *KCNQ1* (del at chr11:2594088). Individual PGM reads contain a high rate of indels in hompolymer stretches, and the Bayesian calling algorithm has been optimised to eliminate these when calling variants in the consensus sequence.

Table 3. Sequencing and target capture performance metrics.

					Alignment						
NGS Platform	Reads	Bases (Mb)	Mean read length	Q20 Bases	Mapped Reads	Reads On Design	Reads On Target	Depth On Target	Evenness	EF	Callable
MiSeq	7757916	889	115	95.8%	90.7%	99.3%	96.7%	1529	68.1%	110111	97.9%
PGM	6133098	969	106	67.5%	100%	96.2%	91.2%	1231	78.8%	104915	96.8%

Mean read length after trimming primer sequences and low quality bases. ReadsOnDesign/ReadsOnTarget = percentage of reads mapping to amplicon design or protein-coding target region. EF = enrichment factor.
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This true deletion (ACCACCCT -> ACCACCT) resembles such an error so, while putative variant alleles were detected by ITS, the variant was rejected as a probable error. This outcome is insensitive to user-defined filter settings.

Of variants located in sequenced regions, 93/93 (100%; 95% CI 98.0–100%) were detected by MiSeq, and 105/106 (99.1%; 95% CI 95.7–99.9%) by the Ion Torrent PGM (Table 6). In a diagnostic setting, the handful of regions of predictable and consistent low coverage (which harboured the missed variants) would be targeted with adjunct Sanger sequencing.

The study was not powered to formally compare indel calling, but 4/4 known indels were detected by MiSeq, and 3/4 by Ion Torrent PGM with one FP.

Resources and Costs

The MiSeq data was obtained with a single sequencing run, at a cost of $959 (£609). It required three Ion Torrent PGM runs to obtain equivalent sequencing output ($686 each) (£439), and one run was repeated due to low bead deposition on the sequencing slide. The total sequencing time (from pooled, barcoded sequencing library to raw sequence data output) was 28 hrs for MiSeq, including one hour of hands-on time. The equivalent time for each Ion Torrent PGM run was shorter (9 hrs), but with 4 hrs hands-on time, as emulsion PCR, enrichment and sequencing occur on separate machines with human intervention at each stage, whereas chip loading and cluster generation are automated on the MiSeq (Table 2).

In summary, PCR-based target enrichment approach followed by MiSeq and Ion Torrent PGM sequencing interrogated 97.9% and 96.8% of the target sufficiently for variant detection with equivalent NGS sequencing output. Variant calling in the regions covered had a PPV of 95.9% (MiSeq, 95% CI: 90.5–98.6%) and 95.5% (PGM, 95% CI: 90.3–98.2%) with sensitivities of 100% (MiSeq, 95% CI: 98.0–100%) and 99.1% (PGM, 95% CI: 95.7–99.9%) (Table 6). In a diagnostic setting, the handful of regions missed are most likely to require adjunct Sanger sequencing to achieve up to 100% sensitivity for the assay as a whole.

Discussion

Assay Coverage

Both platforms achieved very good coverage of the target region. It is unlikely that such an assay will achieve 100% coverage, largely because GC-rich target is difficult to amplify using PCR, both at the target enrichment stage and also during downstream NGS library preparation. We anticipate that for diagnostic use a small number of regions will continue to require conventional sequencing approaches, though such a hybrid

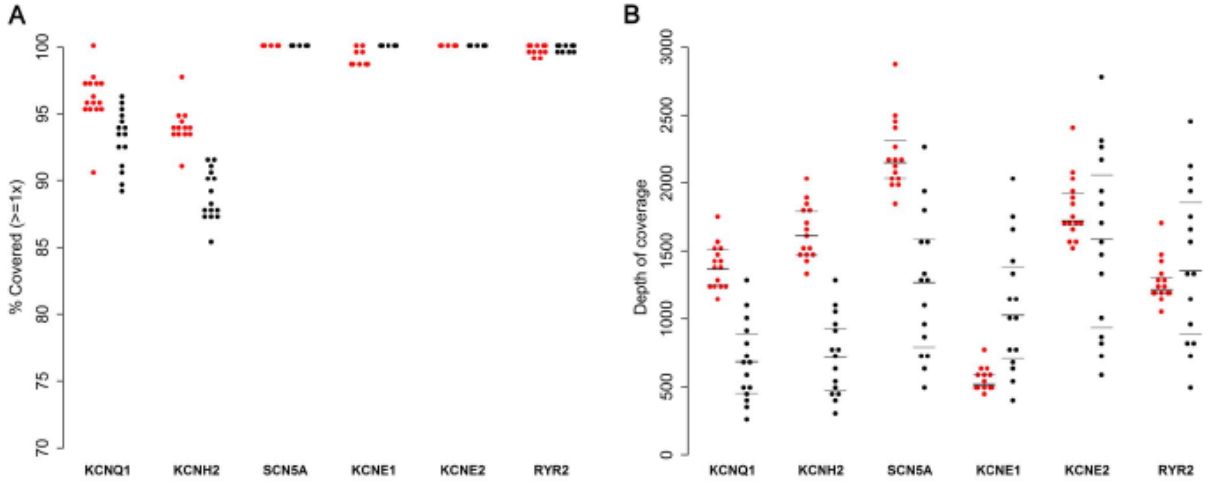

Figure 1. Coverage of target genes. a. The percentage of each gene that is captured and sequenced (at least one read) is shown for each platform (MiSeq in red, PGM in black), for 15 samples; Three genes were consistently fully sequenced. Coverage of KCNQ1 and KCNH2 was more variable: KCNQ1 and KCNE1 were fully covered in the best performing samples, while the best performance on KCNH2 covered >97% of the gene. b. Mean sequencing depth across each gene, for 15 samples. Quartiles are shown. There is significant intra- and inter- sample variability.
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Table 4. Sequencing coverage of each gene.

	MiSeq (%)						PGM (%)					
	1x	10x	20x	30x	50x	100x	1x	10x	20x	30x	50x	100x
KCNE1	98.83	88.77	75.2	69.44	67.64	65.5	100	100	100	100	100	99.81
KCNE2	100	96.42	88.93	85.98	85.98	85.98	100	100	100	100	100	100
KCNH2	94.07	87.66	85.67	84.3	83.22	81.79	88.86	77.91	75.63	74.51	72.95	70.59
KCNQ1	96.22	92.86	89	86.42	79.56	74.03	93.14	86.45	82.68	81.42	80.92	77.39
SCN5A	100	100	99.98	99.69	98.72	95.96	100	100	99.96	99.9	99.75	99.13
RYR2	99.69	97.2	95.16	93.7	90.69	85.78	99.87	99.67	99.65	99.61	99.38	98.86
Overall	98.77	96.14	94.19	92.83	90.3	86.37	97.99	95.98	95.39	95.12	94.72	93.73

The coverage of the protein-coding sequence of each gene of interest is tabulated, as a percentage, for a range of sequencing depths (≥1x, 10x, 20x, 30x, 50x and 100x reads).

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approach still provides for a cost and time saving compared with conventional sequencing. For the six genes studied here, there are 1256 reported disease-causing variants in this protein-coding target region [33]; 1217 (96.9%) fall within the regions covered by the MiSeq, and 1200 (95.5%) by the Ion Torrent PGM.

The Access Array design was iteratively optimized prior to this study (see Methods). The performance of the manufacturer's original amplicon design was assessed, and additional primer pairs added to the assay to improve the capture of regions that were under-represented. This pilot work used the Ion Torrent PGM, as the MiSeq was not available in the UK at that time. This may marginally favour the Ion Torrent PGM: the MiSeq platform performed poorly on amplicons derived from *KCNE1* (chr21:35821729–35821867, Supporting Information Figures S4 and S5), though these were captured by the Access Array. It may be possible to produce MiSeq-compatible amplicons with further iteration tailored to this platform.

These clinically important genes include regions with a very high GC-content (~80%), such as *KCNQ1* exons 1 & 8, *KCNH2* exons 1, 4, 12, and the 3′ portion of exon 2, which perform relatively poorly despite optimization efforts. We have previously found that the performance of some amplicons in the Access Array can be improved using a GC robust PCR mastermix at this stage, but these gains are unlikely to persist if non-GC robust enzymes are used in downstream emulsion PCR during NGS library preparation. However, as Quail *et al* were able to successfully sequence sub-genomic regions with GC-contents >90%, upstream PCR capture, rather than NGS, is likely to be limiting here, and this avenue may still yield further improvements.

An alternative upstream target capture technology might also yield better coverage and hence sensitivity. In this study the capture methodology was fixed to allow unbiased comparison of downstream sequencing, but we have previously compared PCR and hybridisation based approaches for these same gene [34], and found that overall coverage was very similar for both approaches. Other studies have reported reproducible patterns of non-uniform capture across a range of platforms, particular in repetitive sequences and at extremes of GC content [34,35]. In our opinion the choice of upstream target capture is most likely to be driven by cost and capacity requirements: the microfluidic PCR approach employed here is simple, fast and cheap, but has a much smaller capacity than hybridisation approaches, for example.

At the throughput employed in this study, both platforms had significant redundancy of sequencing depth, making them relatively robust to differences in sequencing depth within and between samples. If more samples were processed in a single run to increase throughput, the differences in coverage variability within- and between- samples may become limiting and influence platform choice. Inter-sample variability was most marked when using Ion Torrent PGM, as compared to the MiSeq. Variability between samples (See Figure 1b) is most likely due to stochastic error during pipetting and quantification leading to differences in DNA input at the sequencing stage. In our study there was no evidence of systematic barcode bias where this could be assessed on the PGM. Within sample variability is largely reproducible and sequence-dependent, and is a well-recognized feature of all target enrichment methodologies [34,36], though sequence-dependent bias is present even in whole genome sequencing, without target enrichment.

We acknowledge that we have only studied a small number of genes here, as the assay was matched to the capacity of a PCR-based approach, and intended to reflect a typical clinical assay. Though a range of gene sizes and GC contents were represented, this may limit the generalizability of findings.

Variant Calling

Variant calling was reassuringly accurate. Sensitivity in the regions covered by the assay was excellent with just one variant missed on one platform. Of the four FP SNPs from MiSeq and four FP SNPs from Ion Torrent PGM, one common error in *KCNH2* exon 5 (chr7:150654468G>A) was called on both platforms. This site was deeply sequenced with good allele balance (sequencing depth 2730-fold with 57% alternate reads on MiSeq; sequencing depth 2403-fold with 55% alternate on PGM), good mapping quality and variant detection scores from both platforms. It was the only variant to be discordant between both NGS platforms and the Sanger method, raising the possibility that it is a sequence error introduced by upstream PCR. Five out of six remaining FP SNPs (three MiSeq, two Ion Torrent PGM) were G>A transitions clustered in *KCNH2* exons 12 and 13, and the final Ion Torrent PGM FP was a G>A transition in *SCN5A*. Ion Torrent PGM FPs occurred in regions with good sequencing depth, but significant strand imbalance and noisy sequencing (high base quality in individual reads, but poor consensus between reads). MiSeq FPs were found in areas of relatively low coverage (<100x), with false alternate allele bases found close to the ends of the reads, again with strand imbalance.

Importantly, our pipeline included a custom Perl script to trim poor quality bases at the 3′ end of MiSeq reads. This significantly improved the mapping qualities and reduced the number of false

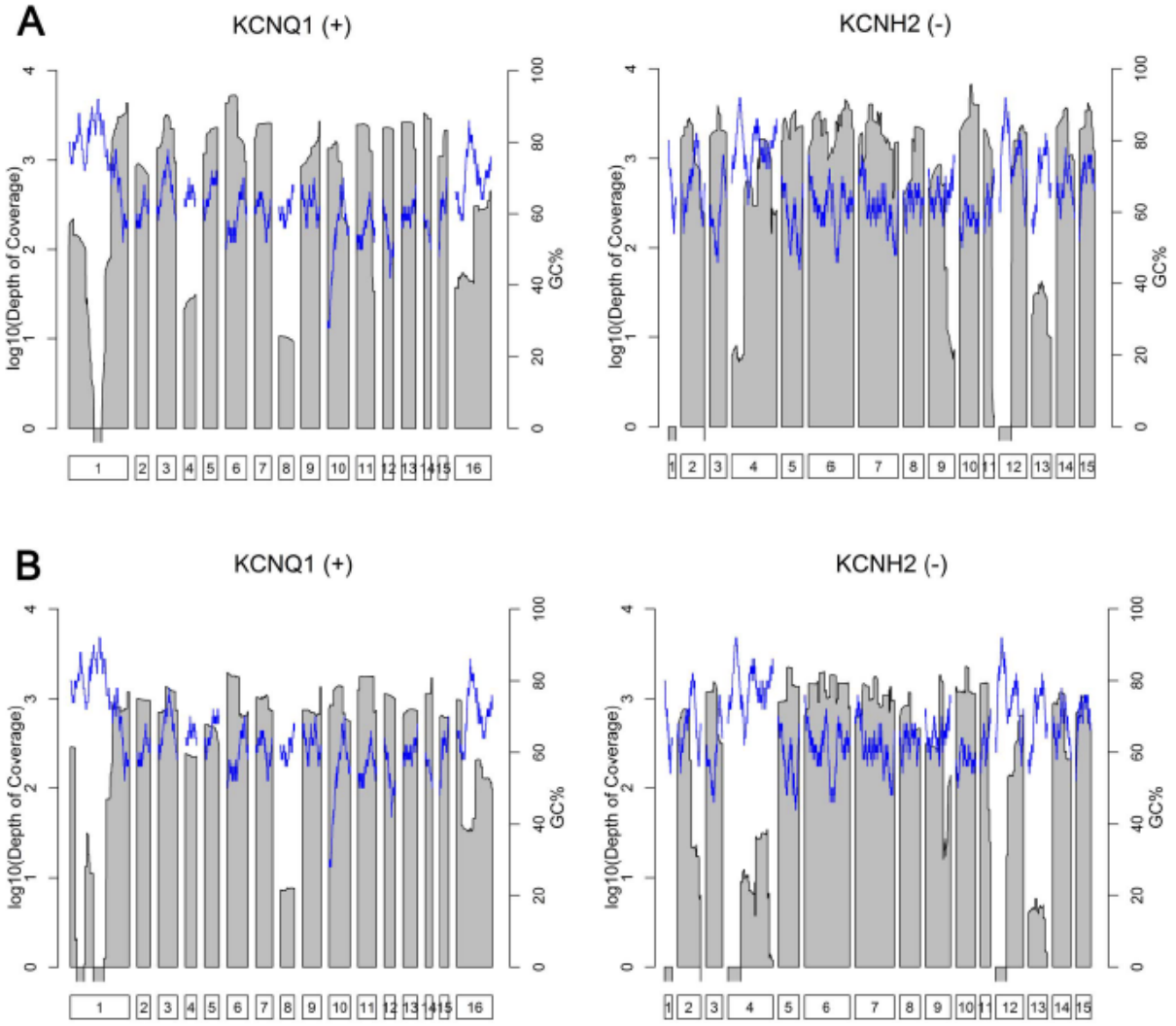

Figure 2. Coverage of KCNQ1 and KCNH2 for the two platforms. Mean depth of coverage for 15 samples is shown for two genes on a log scale. Regions of no coverage therefore have negative values. The blue lines indicate local GC content (calculated with a 50 bp sliding window). Regions consistently missed have high GC content, with similar patterns for both platforms. KCNQ1 exons 1 & 8 and KCNH2 exons 1, 4 & 12 are difficult to sequence. A cartoon of the exon structure is shown beneath each panel. Plus (+) and minus (-) denote gene strand. Plots for all genes are shown in Supporting Information Figure S5. a.) MiSeq b.) Ion Torrent PGM.
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negatives on this platform in our hands (i.e. 9 common variants were rescued which would have otherwise been missed even with depth >400). Analysis of raw reads on both platforms showed a similar substitution mismatch rate (0.5 per 100 bases), with a higher indel rate in homopolymer stretches on the Ion Torrent PGM (1.3 vs 0.02 per 100 bases). Nonetheless, final variant calling accuracy did not differ significantly (odds ratio = 0.90; 95% confidence interval: 0.24–3.46; p-value = 1; Fisher's exact test). This study was not powered to robustly assess differences in indel detection.

The number of PCR amplification cycles used in the two methodological approaches differed slightly. The MiSeq method used 76 PCR amplification cycles, including 26 cycles during flowcell cluster generation, whereas the Ion Torrent PGM used 82 cycles of PCR amplification, including 45 cycles during emulsion PCR. Increasing the number of PCR amplification cycles is known to increase the burden of *Taq*-related errors [37]. There may be room to reduce the number of cycles: for example the manufacturer's protocol for Illumina library preparation uses a small aliquot of diluted template from the Access Array, removing this dilution may allow for fewer PCR cycles.

Current practice in laboratories that are starting to use NGS for clinical applications is to confirm medically actionable variants using Sanger sequencing. This study identified a small but significant number of false positives on both platforms, supporting this practice.

Cost and Time

Given the strong technical performance of both platforms, issues of cost and time are likely to be important to laboratories.

Table 5. Detection of coding variants for each NGS platform.

	Variants detected by dHPLC/Sanger	Variants detected by MiSeq			Variants missed by MiSeq	Variants detected by PGM			Variants missed by PGM
		Total	Concordant with dHPLC/Sanger	NGS only	Total	Concordant with dHPLC/Sanger	NGS only		
Group I (dHPLC)	38	64	32	32	6	74	38	36	0
Group II (Sanger)	36	33	33	0	3	36	34	2	2
Total	74	97	65	32	9	110	72	38	2

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Sequencing capacity and costs change continuously as NGS platforms evolve, but at present the instrument cost of the MiSeq is higher than the Ion Torrent PGM. For a single run, the Ion Torrent PGM was cheaper and faster than the MiSeq, but with more hands-on time and a higher degree of technical complexity. With the throughput used in this study, the final cost per sample was lower for the MiSeq.

The relative youth of the Ion Torrent PGM (UK commercialisation date: mid-2011) means that it is developing rapidly, offering both advantages and challenges to early adopters. Challenges have included rapidly changing laboratory and bioinformatic protocols, reliability issues in our hands, and a modest per-run capacity at this stage. We readily acknowledge that performance on each platform is limited by user experience as well as platform capability, and therefore is likely to continue to improve. Positive developments include the semi-automation of emulsion PCR and bead enrichment, with reduced hands-on time, and the introduction of a larger scale 318-chip, with the potential to match the data output of the MiSeq in a single run. These changes may make the Ion Torrent PGM faster and cheaper overall, though still with more hands-on time than MiSeq. Though we have piloted the 318-scale chip with satisfactory sequencing and quality metrics (data not shown), at the time of data collection for this study we had not yet achieved balanced sequencing of multiple libraries in order to make use of the increased capacity and were continuing to use the 316. Subjectively, the MiSeq (UK commercialisation date: early 2012) has presented a shallower learning curve, with relatively stable protocols and software around the study period. When using the MiSeq platform to sequence low complexity libraries, sequence quality metrics and the number of reads passing bioinformatic filters are noticeably worse than those obtained during high-complexity genome sequencing. Illumina recommend adding 40–50% of a high complexity target (e.g. phi-X bacteriophage genomic DNA) to low complexity PCR-generated libraries at the sample loading stage. This may benefit smaller Access Array-generated libraries, or libraries with fewer samples in the multiplex. Whilst not used for this study, this practice would impact on the total useable yield of the MiSeq platform if widely adopted.

Current diagnostic testing for inherited cardiac arrhythmias in the United Kingdom is limited to a small number of laboratories, using exon PCR and direct Sanger sequencing or first-generation NGS DNA sequencing techniques. We are aware of one UK centre offering NGS analysis of the 5 LQT genes studied here (plus *KCNJ2*) on the Roche 454 GS-FLX sequencer with advertised turnaround time of 40 working days at a cost of $950 (£600) per specimen. The 454 currently produces fewer reads than the desktop sequencers studied here, and the high-throughput target-enrichment approach that we have employed does not require the longer read-lengths that are considered one of the principle advantages of this platform. We conservatively estimate that a diagnostic workflow using multiplex PCR and desktop NGS takes 20 working days to complete (including variant confirmation by Sanger sequencing), with likely cost of less than $630 (£400) per specimen if demand is sufficient to sequence at close to full capacity (full economic cost including DNA extraction, 15-plex testing with MiSeq NGS and Sanger variant confirmation studies). The assay described here also includes the large *RYR2* gene that is associated with another important inherited arrhythmia syndrome, catecholaminergic polymorphic VT (CPVT). *RYR2* is not currently fully sequenced in available clinical assays in the UK: testing is limited to "hotspot" exons (UK Genetic Testing Network, http://www.ukgtn.nhs.uk/, accessed 19th February 2013). A combined assay for LQT & CPVT allows for higher

Table 6. Accuracy of variant calling for NGS platforms.

	Total coding variants	Variant sites interrogated	Variants detected	False positives	Variants missed	Sensitivity	Positive predictive value
MiSeq							
Group I	71	60	60	4	0	100%	93.8%
Group II	36	33	33	0	0	100%	100%
Total	**107**	**93**	**93**	**4**	**0**	**100% (98.0–100)**	**95.9% (90.5–98.6)**
PGM							
Group I	71	71	71	3	0	100%	96.0%
Group II	36	35	34	2	1	97.1%	94.4%
Total	**107**	**106**	**105**	**5**	**1**	**99.1% (95.7–99.9)**	**95.5% (90.3–98.2)**

Group I: dHPLC with Sanger confirmation; Group II: direct Sanger sequencing 95% confidence intervals are given for sensitivity and positive predictive value.
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assay throughput with reduced cost, and is sensible given the phenotypic similarity, the small but important number of *RYR2* mutations reported in "genotype-negative" LQT cases [38], and the value of comprehensive genetic testing in molecular autopsy.

In conclusion, we compared two NGS platforms for diagnostic sequencing. Whilst we do not recommend one platform over another, both are mature technologies for clinical application, with the potential to increase availability of molecular diagnostics in line with national and international recommendations. Performance is promising, though sequence-context and platform-specific biases will influence diagnostic strategies for some genes. Clinical labs should report the coverage of each gene interrogated by such an assay and use conventional methods to cover missed regions and to validate clinically actionable findings. The final choice of platforms is likely to be governed largely by cost and usability.

Accession Numbers

Sequence data has been submitted to the European Nucleotide Archive, accession number ERP002466.

Supporting Information

File S1 ComparisonMiSeq_PGM_supplementary.docx includes six figures and two tables. Figure S1. Characteristics of target capture design: GC content and length of Access Array IFC amplicons. a. Amplicon GC content approximates to a normal distribution 50.3±11.4 (%), <7% amplicons have extreme (>70% or <30%) GC-content. b. Amplicon length (range: 65 bp to 403 bp, median 190 bp and mean 185±29); 85% have a length <200 bp; 98% amplicons have sequence length <240 bp. We used optimised Fluidigm capture to prepare library for Illumina and Ion Torrent platforms (see methods). 386 amplicons, with a combined length of 71,915 bp, are tiled over 47,660 bp of target sequence, of which 27,049 bp is protein coding. **Figure S2. Base quality distributions.** Sequencing base qualities before (left) and after (right) trimming and QC from (a.) MiSeq. (b.) Ion Torrent PGM. The base quality distribution (boxplot at each bar) is plotting against position in the read; the solid-line curve indicates the average base quality. Reads from Ion Torrent PGM have better base quality at 3′end as compared to the raw reads generated by MiSeq. **Figure S3.Readlength distribution.** The read length from MiSeq (a) vary from 20 to 135 bp, with average 115 bp±26 and median 127 bp; Ion Torrent PGM produced up to 267 bp reads (b), with average 106 bp±57 and median 102 bp. **Figure S4. Coverage of target genes.** Here we show the percentage of each target gene that is covered at ≥ x sequencing depth, calculated as a mean across all samples.The lower panels show the same data, with a larger scale on the x-axis. On the PGM, two genes (KCNQ1 & KCHN2) show a sharp drop-off in coverage, suggesting that some regions are difficult to robustly sequence. On the MiSeq, KCNE1 and KCNE2 also showed significant drop-off. **Figure S5. Sequencing coverage of target genes.** Sequencing depth is plotted for each coding base of the six target genes, on a $\log_{10}$ scale. Depth is calculated as a mean across 15 samples. Regions covered by a single read are therefore plotted at the origin, and regions of zero coverage have a negative deflection on the y-axis. GC content (calculated with a 50 bp sliding window on the genomic DNA forward strand) is overlaid in blue. Plus (+) or minus (-) indicates the strand on which each gene is encoded. While some regions are clearly problematic for both platforms (e.g. KCNQ1 exon 2, KCNH2 exons 1 & 12), there are also regions where one platform performs better (e.g. KCNE1, KCNE2, KCNH2 exon 4). **Figure S6. The relationship between GC content and coverage.** Sequencing depth ($\log_{10}$ scale) for each exon is plotted against its GC content. The coefficient of variation is larger for MiSeq than for Ion Torrent PGM (0.931 vs. 0.407). Loess regression is shown in red. MiSeq performance appears more variable across the GC range, whereas Ion Torrent performance falls off at high GC values, perhaps because of the additional emulsion PCR. **Table S1. Barcode indexes and Ion Torrent specific adapters.** Primers used for Ion Torrent PGM barcoded library prep, with index sequences highlighted. Each amplicon is inserted into the complex in both orientations: A-adaptor_Barcode_CommonSequence1_Amplicon_CommonSequence2_P1-adaptor; A-adaptor_Barcode_CommonSequence2_Amplicon_CommonSequence1_P1-adaptor. **Table S2. Detected variant information.** LRG = Locus Reference Genomic; Chr = Chromosome; Ref = reference allele; Alt = Alternative allele; P = Variants revealed by PGM; M = variants revealed by Miseq; Highlighted indicates the SNP was missed by both platforms. Note: All variants appearing in this table were confirmed by Sanger DNA sequencing analysis.
(DOCX)

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Author Contributions

Conceived and designed the experiments: XL AB PB SC JW. Performed the experiments: AB SW LG TN MG TN IV. Analyzed the data: XL RW JW SJ. Wrote the paper: XL AB JW.

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4. Závěr

Navzdory velkým nadějím, které vyvolalo dokončení sekvenace lidského genomu před více než 10 lety, zůstává naše chápání genetického pozadí patofyziologických procesů velmi omezené. Ukazuje se, že architektura genomu není lineární a že stejné sekvence slouží nejen jako předloha pro transkripci ale též jako regulační oblasti. Alely ovlivňující riziko náhlé srdeční smrti tak mohou být přítomny také v tzv. nekódujících oblastech genomu (24). Identifikace takových alel bude úkolem pro tzv. „genome-wide association“ studie a také pro přicházející technologie masové DNA sekvenace. Jakýkoli budoucí genetický screening využitelný pro rizikovou stratifikaci náhlé srdeční smrti by měl zahrnovat hodnocení velkého počtu genomických variant všech oblastí relevantních pro riziko arytmií.

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